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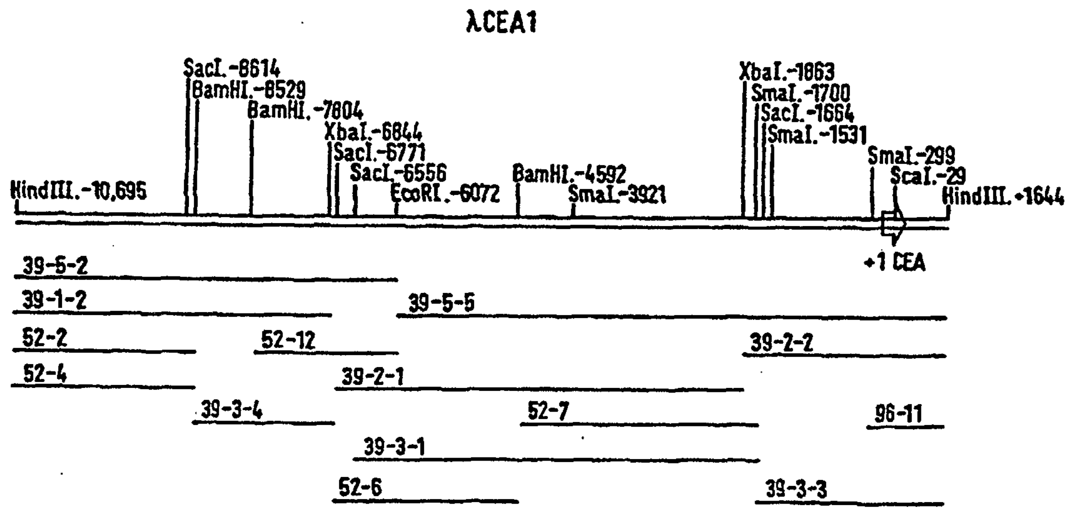


(57) Abstract

The invention relates to the transcriptional regulatory sequence (TRS) of carcinoembryonic antigen (CEA) and molecular chimaera comprising the CEA TRS and DNA encoding a heterologous enzyme. CEA TRS is capable of targeting expression of the heterologous enzyme to CEA+ cells and the heterologous enzyme is preferably an enzyme capable of catalysing the production of an agent cytotoxic or cytostatic to CEA+ cells. For example the enzyme may be cytosine deaminase which is capable of catalysing formation of the cytotoxic compound 5-fluorouracil from the non toxic compound 5-fluorocytosine.

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# TRANSCRIPTIONAL REGULATORY SEQUENCE OF CARCINOEMBRYONIC ANTIGEN FOR EXPRESSION TARGETING

The present invention relates to a transcriptional regulatory sequence useful in gene therapy.

Colorectal carcinoma (CRC) is the second most frequent cancer and the second leading cause of cancer-associated deaths in the United States and Western Europe. The overall five-year survival rate for patients has not meaningfully improved in the last three decades. Prognosis for the CRC cancer patient is associated with the depth of tumor penetration into the bowel wall, the presence of regional lymph node involvement and, most importantly, the presence of distant metastases. The liver is the most common site for distant metastasis and, in approximately 30% of patients, the sole initial site of tumor recurrence after successful resection of the primary colon cancer. Hepatic metastases are the most common cause of death in the CRC cancer patient.

The treatment of choice for the majority of patients with hepatic CRC metastasis is systemic or regional chemotherapy using 5-fluorouracil (5-FU) alone or in combination with other agents such as leviamasole. However, despite extensive effort, there is still no satisfactory treatment for hepatic CRC metastasis. Systemic single- and combination-agent chemotherapy and radiation are relatively ineffective emphasizing the need for new approaches and therapies for the treatment of the diseases.

A gene therapy approach is being developed for primary and metastatic liver tumors that exploits the transcriptional differences between normal and metastatic cells. This approach involves linking the transcriptional regulatory sequences (TRS) of a tumor associated marker gene to the coding sequence of an enzyme, typically a non-

mammalian enzyme, to create an artificial chimaeric gene that is selectively expressed in cancer cells. The enzyme should be capable of converting a non-toxic prodrug into a cytotoxic or cytostatic drug thereby allowing for selective elimination of metastatic cells.

The principle of this approach has been demonstrated using an alpha-fetoprotein/Varicella Zoster virus thymidine kinase chimaera to target hepatocellular carcinoma with the enzyme metabolically activating the non-toxic prodrug 6-methoxypurine arabinonucleoside ultimately leading to formation of the cytoxic anabolite adenine arabinonucleoside triphosphate (see Huber et al, Proc. Natl. Acad. Sci U.S.A., 88, 8039-8043 (1991) and EP-A-0 415 731).

For the treatment of hepatic metastases of CRC, it is desirable to control the expression of an enzyme with the transcriptional regulatory sequences of a tumor marker associated with such metastases.

CEA is a tumor associated marker that is regulated at the transcriptional level and is expressed by most CRC tumors but is not expressed in normal liver. CEA is widely used as an important diagnostic tool for postoperative surveillance, chemotherapy efficacy determinations, immunolocalisation and immunotherapy. The TRS of CEA are potentially of value in the selective expression of an enzyme in $CEA^+$ tumor cells since there appears to be a very low heterogeneity of CEA within metastatic tumors, perhaps because CEA may have an important functional role in metastasis.

The cloning of the CEA gene has been reported and the promoter localised to a region of 424 nucleotides upstream from the translati nal start (Schrewe *et al*, Mol. Cell. Biol., 10, 2738 - 2748 (1990) but the full TRS was not

identified.

In the work on which the present invention is based, CEA genomic clones have been identified and isolated from the human chromosome 19 genomic library LL19NL01, ATCC number 57766, by standard techniques described hereinafter. The cloned CEA sequences comprise CEA enhancers in addition to the CEA promoter. The CEA enhancers are especially advantageous for high level expression in CEA-positive cells and no expression in CEA-negative cells.

According to one aspect, the present invention provides a DNA molecule comprising the CEA TRS but without associated CEA coding sequence.

According to another aspect, the present invention provides use of a CEA TRS for and targeting expression of a heterologous enzyme to $CEA^+$ cells. Preferably the enzyme is capable of catalysing the production of an agent cytotoxic or cytostatic to the $CEA^+$ target cells.

As described in more detail hereinafter, the present inventors have sequenced a large part of the CEA gene upstream of the coding sequence. As used herein, the term "CEA TRS" means any part of the CEA gene upstream of the coding sequence which has a transcriptional regulatory effect on a heterologous coding sequence operably linked thereto.

Certain parts of the sequence of the CEA gene upstream of the coding sequence have been identified as making significant contributions to the transcriptional regulatory

effect, more particularly increasing the level and/or selectivity of transcription. Preferably the CEA TRS includes all or part of the region from about -299b to about +69b, more preferably about -90b to about +69b. Increases in the level of transcription and/or selectivity can also be obtained by including one or more of the following regions: -14.5kb to -10.6kb, preferably -13.6kb to -10.6kb, and/or -6.1kb to -3.8kb. All of the regions referred to above can be included in either orientation and in different combinations. In addition, repeats of these regions may be included, particularly repeats of the -90b to +69b region, containing for example 2,3, 4 or more copies of the region. The base numbering refers to the sequence of Figure 6. The regions referred to are included in the plasmids described in figure 5B.

Gene therapy involves the stable integration of new genes into target cells and the expression of those genes, once they are in place, to alter the phenotype of that particular target cell (for review see Anderson, W.F. Science 226, 401-409 (1984) and McCormick, D. Biotechnology 3, 689-693, (1985)). Gene therapy may be beneficial for the treatment of genetic diseases that involve the replacement of one defective or missing enzyme, such as; hypoxanthine-guanine phosphoribosyl transferase in Lesch-Nyhan disease, purine nucleoside phosphorylase in severe immunodeficiency disease, and adenosine deaminase in severed combined immunodeficiency disease.

It has now been found that it is possible to selectively arrest the growth of, or kill, mammalian carcinoma cells with prodrugs, i.e. chemical agents capable

of selective conversion to cytotoxic (causing cell death) or cytostatic (suppressing cell multiplication and growth) metabolites. This is achieved by the construction of a molecular chimaera comprising a "target tissue-specific" TRS that is selectively activated in target cells, such as cancerous cells, and that controls the expression of a heterologous enzyme. This molecular chimaera may be manipulated via suitable vectors and incorporated into an infective virion. Upon administration of an infective virion containing the molecular chimaera to a host (e.g., mammal or human), the enzyme is selectively expressed in the target cells. Administration of prodrugs (compounds that are selectively metabolised by the enzyme into metabolites that are either further metabolised to or are, in fact, cytotoxic or cytostatic agents) can then result in the production of the cytotoxic or cytostatic agent <u>in situ</u> in the cancer cell. According to the present invention CEA TRS provides the target tissue specificity.

Molecular chimaeras (recombinant molecules comprised of unnatural combinations of genes or sections of genes), and infective virions (complete viral particles capable of infecting appropriate host cells) are well known in the art of molecular biology.

A number of enzyme prodrug combinations may be used for the above purpose, providing the enzyme is capable of selectively activating the administered compound either directly or through an intermediate to a cytostatic or cytotoxic metabolite. The choice of compound will also depend on the enzyme system used, but must be selectively metabolised by the enzyme either directly or indirectly to a cytotoxic or cytostatic metabolite. The term heterologous enzyme, as used herein, refers to an enzyme that is derived from or associated with a species which is different from the host to be treated and which will display the appropriate characteristics of the abov

mentioned selectivity. In addition, it will also be appreciated that a heterologous enzyme may also refer to an enzyme that is derived from the host to be treated that has been modified to have unique characteristics unnatural to the host.

The enzyme cytosine deaminase (CD) catalyses the deamination of cytosine to uracil. Cytosine deaminase is present in microbes and fungi but absent in higher eukaryotes. This enzyme catalyses the hydrolytic deamination of cytosine and 5-fluorocytosine (5-FC) to uracil and 5-fluorouracil (5-FU), respectively. Since mammalian cells do not express significant amounts of cytosine deaminase, they are incapable of converting 5-FC to the toxic metabolite 5-FU and therefore 5-fluorocytosine is nontoxic to mammalian cells at concentrations which are effective for antimicrobial activity. 5-Fluorouracil is highly toxic to mammalian cells and is widely used as an anticancer agent.

In mammalian cells, some genes are ubiquitously expressed. Most genes, however, are expressed in a temporal and/or tissue-specific manner, or are activated in response to extracellular inducers. For example, certain genes are actively transcribed only at very precise times in ontogeny in specific cell types, or in response to some inducing stimulus. This regulation is mediated in part by the interaction between transcriptional regulatory sequences (for example, promoter and enhancer regulatory DNA sequences), and sequence-specific, DNA-binding transcriptional protein factors.

It has now been found that it is possible to alter certain mammalian cells, e.g. colorectal carcinoma cells, metastatic colorectal carcinoma cells and hepatic colorectal carcinoma cells to selectively express a heterologous enzyme as hereinbefore defined, e.g. CD. This

is achieved by the construction of molecular chimaeras in an expression cassette.

Expression cassettes themselves are well known in the art of molecular biology. Such an expression cassette contains all essential DNA sequences required for expression of the heterologous enzyme in a mammalian cell. For example, a preferred expression cassette will contain a molecular chimaera containing the coding sequence for CD, an appropriate polyadenylation signal for a mammalian gene (i.e., a polyadenylation signal that will function in a mammalian cell), and CEA enhancers and promoter sequences in the correct orientation.

Normally, two DNA sequences are required for the complete and efficient transcriptional regulation of genes that encode messenger RNAs in mammalian cells: promoters and enhancers. Promoters are located immediately upstream (5′) from the start site of transcription. Promoter sequences are required for accurate and efficient initiation of transcription. Different gene-specific promoters reveal a common pattern of organisation. A typical promoter includes an AT-rich region called a TATA box (which is located approximately 30 base pairs 5′ to the transcription initiation start site) and one or more upstream promoter elements (UPEs). The UPEs are a principle target for the interaction with sequence-specific nuclear transcriptional factors. The activity of promoter sequences is modulated by other sequences called enhancers. The enhancer sequence may be a great distance from the promoter in either an upstream (5′) or downstream (3′) position. Hence, enhancers operate in an orientation- and position-independent manner. However, based on similar structural organisation and function that may be interchanged, the absolute distinction between promoters and enhancers is somewhat arbitrary. Enhancers increase the rate of transcription from the promoter sequence. It

is predominantly the interaction between sequence-specific transcriptional factors with the UPE and enhancer sequences that enable mammalian cells to achieve tissue-specific gene expression. The presence of these transcriptional protein factors (tissue-specific, trans-activating factors) bound to the UPE and enhancers (cis-acting, regulatory sequences) enables other components of the transcriptional machinery, including RNA polymerase, to initiate transcription with tissue-specific selectivity and accuracy.

The transcriptional regulatory sequence for CEA is suitable for targeting expression in colorectal carcinoma, metastatic colorectal carcinoma, and hepatic colorectal metastases, transformed cells of the gastrointestinal tract, lung, breast and other tissues. By placing the expression of the gene encoding CD under the transcriptional control of the CRC-associated marker gene, CEA, the nontoxic compound, 5-FC, can be metabolically activated to 5-FU selectively in CRC cells (for example, hepatic CRC cells). An advantage of this system is that the generated toxic compound, 5-fluorouracil, can diffuse out of the cell in which it was generated and kill adjacent tumor cells which did not incorporate the artificial gene for cytosine deaminase.

In the work on which the present invention is based, CEA genomic clones were identified and isolated from the human chromosome 19 genomic library LL19NL01, ATCC number 57766, by standard techniques described hereinafter. The cloned CEA sequences comprise CEA enhancers in addition to the CEA promoter. The CEA enhancers are especially advantageous for high level expression in CEA-positive cells and no expression in CEA-negative cells.

The present invention further provides a molecular chimaera comprising a CEA TRS and a DNA sequence operatively linked thereto encoding a heterologous enzyme,

preferably an enzyme capable of catalysing the production of an agent cytotoxic or cytostatic to the $CEA^+$ cells.

The present invention further provides a molecular chimaera comprising a DNA sequence containing the coding sequence of the gene that codes for a heterologous enzyme under the control of a CEA TRS in an expression cassette.

The present invention further provides in a preferred embodiment a molecular chimaera comprising a CEA TRS which is operatively linked to the coding sequence for the gene encoding a non-mammalian cytosine deaminase (CD). The molecular chimaera comprises a promoter and additionally comprises an enhancer.

In particular, the present invention provides a molecular chimaera comprising a DNA sequence of the coding sequence of the gene coding for the heterologous enzyme, which is preferably CD, additionally including an appropriate polyadenylation sequence, which is linked downstream in a 3′ position and in the proper orientation to a CEA TRS. Most preferably the expression cassette also contains an enhancer sequence.

Preferably non-mammalian CD is selected from the group consisting of bacterial, fungal, and yeast cytosine deaminase.

The molecular chimaera of the present invention may be made utilizing standard recombinant DNA techniques.

Another aspect of the invention is the genomic CEA sequence as described by Seq ID1.

The coding sequence of CD and a polyadenylation signal (for example see S q IDs 1 and 2) are placed in the proper 3′ orientation to the essential CEA transcriptional

regulatory elements. This molecular chimaera enables the selective expression of CD in cells or tissue that normally express CEA. Expression of the CD gene in mammalian CRC and metastatic CRC (hepatic colorectal carcinoma metastases) will enable nontoxic 5-FC to be selectively metabolised to cytotoxic 5-FU.

Accordingly, in a another aspect of the present invention, there is provided a method of constructing a molecular chimaera comprising linking a DNA sequence encoding a heterologous enzyme gene, e.g. CD, to a CEA TRS.

In particular the present invention provides a method of constructing a molecular chimaera as herein defined, the method comprising ligating a DNA sequence encoding the coding sequence and polyadenylation signal of the gene for a heterologous enzyme (e.g. non-mammalian CD) to a CEA TRS (e.g., promoter sequence and enhancer sequence).

These molecular chimaeras can be delivered to the target tissue or cells by a delivery system. For administration to a host (e.g., mammal or human), it is necessary to provide an efficient *in vivo* delivery system that stably incorporates the molecular chimaera into the cells. Known methods utilize techniques of calcium phosphate transfection, electroporation, microinjection, liposomal transfer, ballistic barrage, DNA viral infection or retroviral infection. For a review of this subject see Biotechniques 6, No.7, (1988).

The technique of retroviral infection of cells to integrate artificial genes employs retroviral shuttle vectors which are known in the art (Miller A.D., Buttimore C. Mol. Cell. Biol. 6, 2895-2902 (1986)). Essentially, retroviral shuttle vectors (retroviruses comprising molecular chimaeras used to deliver and stably integrate the molecular chimaera into the genome of the target cell)

are generated using the DNA form of the retrovirus contained in a plasmid. These plasmids also contain sequences necessary for selection and growth in bacteria. Retroviral shuttle vectors are constructed using standard molecular biology techniques well known in the art. Retroviral shuttle vectors have the parental endogenous retroviral genes (e.g., gag, pol and env) removed from the vectors and the DNA sequence of interest is inserted, such as the molecular chimaeras that have been described. The vectors also contain appropriate retroviral regulatory sequences for viral encapsidation, proviral insertion into the target genome, message splicing, termination and polyadenylation. Retroviral shuttle vectors have been derived from the Moloney murine leukaemia virus (Mo-MLV) but it will be appreciated that other retroviruses can be used such as the closely related Moloney murine sarcoma virus. Other DNA viruses may also prove to be useful as delivery systems. The bovine papilloma virus (BPV) replicates extrachromosomally, so that delivery systems based on BPV have the advantage that the delivered gene is maintained in a nonintegrated manner.

Thus according to a further aspect of the present invention there is provided a retroviral shuttle vector comprising the molecular chimaeras as hereinbefore defined.

The advantages of a retroviral-mediated gene transfer system are the high efficiency of the gene delivery to the targeted tissue or cells, sequence specific integration regarding the viral genome (at the 5′ and 3′ long terminal repeat (LTR) sequences) and little rearrangements of delivered DNA compared to other DNA delivery systems.

Accordingly in a preferred embodiment of the present invention there is provided a retroviral shuttle vector comprising a DNA sequence comprising a 5′ viral LTR

sequence, a cis-acting psi-encapsidation sequence, a molecular chimaera as hereinbefore defined and a 3' viral LTR sequence.

In a preferred embodiment, and to help eliminate non-tissue-specific expression of the molecular chimaera, the molecular chimaera is placed in opposite transcriptional orientation to the 5' retroviral LTR. In addition, a dominant selectable marker gene may also be included that is transcriptionally driven from the 5' LTR sequence. Such a dominant selectable marker gene may be the bacterial neomycin-resistance gene NEO (aminoglycoside 3' phosphotransferase type II), which confers on eukaryotic cells resistance to the neomycin analogue Geneticin (antibiotic G418 sulphate; registered trademark of GIBCO). The NEO gene aids in the selection of packaging cells that contain these sequences.

The retroviral vector is preferably based on the Moloney murine leukaemia virus but it will be appreciated that other vectors may be used. Vectors containing a NEO gene as a selectable marker have been described, for example, the N2 vector (Eglitis M.A., Kantoff P., Gilboa E., Anderson W.F. Science 230, 1395-1398 (1985)).

A theoretical problem associated with retroviral shuttle vectors is the potential of retroviral long terminal repeat (LTR) regulatory sequences transcriptionally activating a cellular oncogene at the site of integration in the host genome. This problem may be diminished by creating SIN vectors. SIN vectors are self-inactivating vectors that contain a deletion comprising the promoter and enhancer regions in the retroviral LTR. The LTR sequences of SIN vectors do not transcriptionally activate 5' or 3' genomic sequences. The transcriptional inactivation of the viral LTR sequences diminishes insertional activation of adjacent target cell

DNA sequences and also aids in the selected expression of the delivered molecular chimaera. SIN vectors are created by removal of approximately 299 bp in the 3' viral LTR sequence (Gilboa E., Eglitis P.A., Kantoff P.W., Anderson W.F. Biotechniques 4, 504-512 (1986)).

Thus preferably the retroviral shuttle vectors of the present invention are SIN vectors.

Since the parental retroviral *gag*, *pol*, and *env* genes have been removed from these shuttle vectors, a helper virus system may be utilised to provide the *gag*, *pol*, and *env* retroviral gene products *in trans* to package or encapsidate the retroviral vector into an infective virion. This is accomplished by utilising specialised "packaging" cell lines, which are capable of generating infectious, synthetic virus yet are deficient in the ability to produce any detectable wild-type virus. In this way the artificial synthetic virus contains a chimaera of the present invention packaged into synthetic artificial infectious virions free of wild-type helper virus. This is based on the fact that the helper virus that is stably integrated into the packaging cell contains the viral structural genes, but is lacking the psi-site, a cis-acting regulatory sequence which must be contained in the viral genomic RNA molecule for it to be encapsidated into an infectious viral particle.

Accordingly, in a still further aspect of the present invention, there is provided an infective virion comprising a retroviral shuttle vector, as hereinbefore described, said vector being encapsidated within viral proteins to create an artificial, infective, replication-defective, retrovirus.

In a another aspect of the pr sent invention there is provided a method for producing infective virions of the

present invention by delivering the artificial retroviral shuttle vector comprising a molecular chimaera of the invention, as hereinbefore described, into a packaging cell line.

The packaging cell line may have stably integrated within it a helper virus lacking a psi-site and other regulatory sequence, as hereinbefore described, or, alternatively, the packaging cell line may be engineered so as to contain helper virus structural genes within its genome. In addition to removal of the psi-site, additional alterations can be made to the helper virus LTR regulatory sequences to ensure that the helper virus is not packaged in virions and is blocked at the level of reverse transcription and viral integration. Alternatively, helper virus structural genes (i.e., gag, pol, and env) may be individually and independently transferred into the packaging cell line. Since these viral structural genes are separated within the packaging cell's genome, there is little chance of covert recombinations generating wild-type virus.

The present invention also provides a packaging cell line comprising an infective virion, as described hereinbefore, said virion further comprising a retroviral shuttle vector.

The present invention further provides for a packaging cell line comprising a retroviral shuttle vector as described hereinbefore.

In addition to retroviral-mediated gene delivery of the chimeric, artificial, therapeutic gene, other gene delivery systems known to those skilled in the art can be used in accordance with the present invention. These other gene delivery systems include other viral gene delivery systems known in the art, such as the adenovirus delivery

systems.

Non-viral delivery systems can be utilized in accordance with the present invention as well. For example, liposomal delivery systems can deliver the therapeutic gene to the tumor site via a liposome. Liposomes can be modified to evade metabolism and/or to have distinct targeting mechanisms associated with them. For example, liposomes which have antibodies incorporated into their structure, such as antibodies to CEA, can have targeting ability to CEA-positive cells. This will increase both the selectivity of the present invention as well as its ability to treat disseminated disease (metastasis).

Another gene delivery system which can be utilized according to the present invention is receptor-mediated delivery, wherein the gene of choice is incorporated into a ligand which recognizes a specific cell receptor. This system can also deliver the gene to a specific cell type. Additional modifications can be made to this receptor-mediated delivery system, such as incorporation of adenovirus components to the gene so that the gene is not degraded by the cellular lysosomal compartment after internalization by the receptor.

The infective virion or the packaging cell line according to the invention may be formulated by techniques well known in the art and may be presented as a formulation (composition) with a pharmaceutically acceptable carrier therefor. Pharmaceutically acceptable carriers, in this instance physiologic aqueous solutions, may comprise liquid medium suitable for use as vehicles to introduce the infective virion into a host. An example of such a carrier is saline. The infective virion or packaging cell line may be a solution or suspension in such a vehicle. Stabilizers and antioxidants and/or other excipients may also be

present in such pharmaceutical formulations (compositions), which may be administered to a mammal by any conventional method (e.g., oral or parenteral routes). In particular, the infective virion may be administered by intra-venous or intra-arterial infusion. In the case of treating hepatic metastatic CRC, intra-hepatic arterial infusion may be advantageous. The packaging cell line can be administered directly to the tumor or near the tumor and thereby produce infective virions directly at or near the tumor site.

Accordingly, the present invention provides a pharmaceutical formulation (composition) comprising an infective virion or packaging cell line according to the invention in admixture with a pharmaceutically acceptable carrier.

Additionally, the present invention provides methods of making pharmaceutical formulations(compositions), as herein described, comprising mixing an artificial infective virion, containing a molecular chimaera according to the invention as described hereinbefore, with a pharmaceutically acceptable carrier.

The present invention also provides methods of making pharmaceutical formulations (compositions), as herein described, comprising mixing a packaging cell line, containing an infective virion according to the invention as described hereinbefore, with a pharmaceutically acceptable carrier.

Although any suitable compound that can be selectively converted to a cytotoxic or cytotostatic metabolite by the enzyme cytosine deaminase may be utilised, the preferred compound for use according to the invention is 5-FC, in particular for use in treating colorectal carcinoma (CRC), metastatic colorectal carcinoma, or hepatic CRC metastases. 5-FC, which is non-toxic and is used as an antifungal, is

converted by CD into the established cancer therapeutic 5-FU.

Any agent that can potentiate the antitumor effects of 5-FU can also potentiate the antitumor effects of 5-FC since, when used according to the present invention, 5-FC is selectively converted to 5-FU. According to another aspect of the present invention, agents such as leucovorin and levemisol, which can potentiate the antitumor effects of 5-FU, can also be used in combination with 5-FC when 5-FC is used according to the present invention. Other agents which can potentiate the antitumor effects of 5-FU are agents which block the metabolism 5-FU. Examples of such agents are 5-substituted uracil derivatives, for example, 5-ethynyluracil and 5-bromvinyluracil (PCT/GB91/01650 (WO 92/04901); Cancer Research 46, 1094, (1986) which are incorporated herein by reference in their entirety). Therefore, a further aspect of the present invention is the use of an agent which can potentiate the antitumor effects of 5-FU, for example, a 5-substituted uracil derivative such as 5-ethynyluracil or 5-bromvinyluracil in combination with 5-FC when 5-FC is used according to the present invention. The present invention further includes the use of agents which are metabolised *in vivo* to the corresponding 5-substituted uracil derivatives described hereinbefore (see Biochemical Pharmacology 38, 2885, (1989) which is incorporated herein by reference in its entirety) in combination with 5-FC when 5-FC is used according to the present invention.

5-FC is readily available (e.g., United States Biochemical, Sigma) and well known in the art. Leucovorin and levemisol are also readily available and well known in the art.

Two significant advantages of the enzyme/prodrug combination of cytosine deaminase/5-fluorocytosine and

further aspects of the invention are the following:

1. The metabolic conversion of 5-FC by CD produces 5-FU which is the drug of choice in the treatment of many different types of cancers, such as colorectal carcinoma.

2. The 5-FU that is selectively produced in one cancer cell can diffuse out of that cell and be taken up by both non-facilitated diffusion and facilitated diffusion into adjacent cells. This produces a neighbouring cell killing effect. This neighbour cell killing effect alleviates the necessity for delivery of the therapeutic molecular chimera to every tumor cell. Rather, delivery of the molecular chimera to a certain percentage of tumor cells can produce the complete eradication of all tumor cells.

The amounts and precise regimen in treating a mammal, will of course be the responsibility of the attendant physician, and will depend on a number of factors including the type and severity of the condition to be treated. However, for hepatic metastatic CRC, an intrahepatic arterial infusion of the artificial infective virion at a titer of between 2 x $10^5$ and 2 x $10^7$ colony forming units per ml (CFU/ml) infective virions is suitable for a typical tumour. Total amount of virions infused will be dependent on tumour size and are preferably given in divided doses.

Likewise, the packaging cell line is administered directly to a tumor in an amount of between 2 x $10^5$ and 2 x $10^7$ cells. Total amount of packaging cell line infused will be dependent on tumour size and is preferably given in divided doses.

Prodrug treatment - Subsequent to infection with the infective virion, certain cytosine compounds (prodrugs of 5-FU) are converted by CD to cytoxic or cytostatic metabolites (e.g. 5-FC is converted to 5-FU) in target

cells. The above mentioned prodrug compounds are administered to the host (e.g. mammal or human) between six hours and ten days, preferably between one and five days, after administration of the infective virion.

The dose of 5-FC to be given will advantageously be in the range 10 to 500 mg per kg body weight of recipient per day, preferably 50 to 500 mg per kg bodyweight of recipient per day, more preferably 50 to 250 mg per kg bodyweight of recipient per day, and most preferably 50 to 150 mg per kg body weight of recipient per day. The mode of administration of 5-FC in humans are well known to those skilled in the art. Oral administration and/or constant intravenous infusion of 5-FC are anticipated by the instant invention to be preferable.

The doses and mode of administration of leucovorin and levemisol to be used in accordance with the present invention are well known or readily determined by those clinicians skilled in the art of oncology.

The dose and mode of administration of the 5-substituted uracil derivatives can be determined by the skilled oncologist. Preferably, these derivatives are given by intravenous injection or orally at a dose of between 0.01 to 50 mg per kg body weight of the recipient per day, particularly 0.01 to 10 mg per kg body weight per day, and more preferably 0.01 to 0.4 mg per kg bodyweight per day depending on the derivative used. An alternative preferred administration regime is 0.5 to 10 mg per kg body weight of recipient once per week.

The following examples serve to illustrate the present invention but should not be construed as a limitation thereof. In the Examples reference is made to the Figures a brief description of which is as follows:

Figure 1: Diagram of CEA phage clones. The overlapping clones lambdaCEA1, lambdaCEA7, and lambdaCEA5 represent an approximately 26 kb region of CEA genomic sequence. The 11,288 bp HindIII-Sau3A fragment that was sequenced is represented by the heavy line under lambdaCEA1. The 3774 bp HindIII-HindIII fragment that was sequenced is represented by the heavy line under lambdaCEA7. The bent arrows represent the transcription start point for CEA mRNA. The straight arrows represent the oligonucleotides CR15 and CR16. H, HindIII; S, SstI; B, BamHI; E, EcoRI; X, XbaI.

Figure 2: Restriction map of part of lambdaCEA1. The arrow head represents the approximate location of the transcription imitation point for CEA mRNA. Lines below the map represent the CEA inserts of pBS+ subclones. These subclones are convenient sources for numerous CEA restriction fragments.

DNA sequence of the 11,288 bp HindIII to Sau3A fragment of lambdaCEA7 (SEQ ID NO: 1). Sequence is numbered with the approximate transcription imitation point for CEA mRNA as 0 (this start site is approximate because there is some slight variability in the start site among individual CEA transcripts). The translation of the first exon is shown. Intron 1 extends from +172 to beyond +592. Several restriction sites are shown above the sequence. In subclone 109-3 the sequence at positions +70 has been altered by site-directed mutagenesis in order to introduce HindIII and EcoRI restriction sites.

DNA sequence of the 3774 bp Hind III to HindIII fragment of lambda CEA7 (SEQ ID NO: 2).

Figure 3 : Mapplot of 15,056 bp HindIII to Sau3A fragment from CEA genomic DNA showing consensus sequences.

Schematic representation of some of the consensus sequences found in the CEA sequence of Seq IDs 1 and 2. The consensus sequences shown here are from the transcriptional dictionary of Locker and Buzard (DNA Sequence 1, 3-11 (1990)). The lysozymal silencer is coded B18. The last line represents 90% homology to the topoisomerase II cleavage consensus.

Figure 4: Cloning scheme for CEA constructs extending from -299 bp to +69 bp.

Figure 5A: Cloning scheme for CEA constructs extending from -10.7 kb to +69 bp.

Figure 5B: Coordinates for CEA sequence present in several CEA/luciferase clones. CEA sequences were cloned into the multiple cloning region of pGL2-Basic (Promega Corp.) by standard techniques.

Figures 5C and 5D: Transient luciferase assays. Transient transfections and luciferase assays were performed in quadruplicate by standard techniques using DOTAP (Boehringer Mannheim, Indianapolis, IN, USA), luciferase assay system (Promega, Madison, WI, USA), and Dynatech luminometer (Chantilly, VA, USA). CEA-positive cell lines included LoVo (ATCC #CCL 229) and SW1463 (ATCC #CCL 234). CEA-negative cell lines included HuH7 and Hep3B (ATCC #HB 8064). C. Luciferase activity expressed as the percent of pGL2-Control plasmid activity. D. Luciferase activities of LoVo and SW1463 expressed as fold increase over activity in Hep3B.

Example 1

Construction of transcriptional regulatory sequence of carcinoembryonic antigen/cytosine deaminase molecular

chimaera

A) Cloning and isolation of the transcriptional regulatory sequence of the carcinoembryonic antigen gene

CEA genomic clones were identified and isolated from the human chromosome 19 genomic library LL19NL01, ATCC #57766, by standard techniques (Richards et al., Cancer Research, 50, 1521-1527 (1990) which is herein incorporated by reference in its entirety). The CEA clones were identified by plaque hybridization to $^{32}P$ end-labelled oligonucleotides CR15 and CR16. CR15, 5'-CCCTGTGATCTCCAGGACAGCTCAGTCTC-3' (SEQ ID NO: 3), and CR16, 5'-GTTTCCTGAGTGATGTCTGTGTGCAATG-3' (SEQ ID NO: 4), hybridize to a 5' non-transcribed region of CEA that has little homology to other members of the CEA gene family. Phage DNA was isolated from three clones that hybridized to both oligonucleotide probes. Polymerase chain reaction, restriction mapping, and DNA sequence analysis confirmed that the three clones contained CEA genomic sequences. The three clones are designated lambdaCEA1, lambdaCEA5, and lambdaCEA7 and have inserts of approximately 13.5, 16.2, and 16.7 kb respectively. A partial restriction map of the three overlapping clones is shown in Figure 1.

Clone lambdaCEA1 was initially chosen for extensive analysis. Fragments isolated from lambdaCEA1 were subcloned using standard techniques into the plasmid pBS+ (Stratagene Cloning Systems, La Jolla, CA, USA) to facilitate sequencing, site-directed mutagenesis, and construction of chimeric genes. The inserts of some clones are represented in Figure 2. The complete DNA sequence of a 11,288 bp HindIII/Sau3A restriction fragment from lambdaCEA1 (SEQ ID NO: 1) was determined by the dideoxy sequencing method using the dsDNA Cycle Sequencing System from Life Technologies, Inc. and multiple oligonucleotide primers. This sequence extends from -10.7 kb to +0.6 kb relative to

the start site of CEA mRNA. The sequence of 3774 base pair HindIII restriction fragment from lambdaCEA1 was also determined ( SEQ ID NO: 2). This sequence extends from -14.5 kb to -10.7 kb relative to the start site of CEA mRNA. This HindIII fragment is present in plasmid pCR145.

To determine important transcriptional regulatory sequences various fragments of CEA genomic DNA are linked to a reporter gene such as luciferase or chloramphenicol acetyltransferase. Various fragments of CEA genomic DNA are tested to determine the optimized, cell-type specific TRS that results in high level reporter gene expression in CEA-positive cells but not in CEA-negative cells. The various reporter constructs, along with appropriate controls, are transfected into tissue culture cell lines that express high, low, or no CEA. The reporter gene analysis identifies both positive and negative transcriptional regulatory sequences. The optimized CEA-specific TRS is identified through the reporter gene analysis and is used to specifically direct the expression of any desired linked coding sequence, such as CD or VZV TK, in cancerous cells that express CEA. The optimized CEA-specific TRS, as used herein, refers to any DNA construct that directs suitably high levels of expression in CEA positive cells and low or no expression in CEA-negative cells. The optimized CEA-specific TRS consists of one or several different fragments of CEA genomic sequence or multimers of selected sequences that are linked together by standard recombinant DNA techniques. It will be appreciated by those skilled in the art that the optimized CEA-specific TRS may also include some sequences that are not derived from the CEA genomic sequences shown in Seq IDs 1 and 2. These other sequences may include sequences from adjoining regions of the CEA locus, such as sequences from the introns, or sequences further upstream or downstream from the sequenced DNA shown in Seq IDs 1 and 2, or they could include transcriptional control elements from other genes that when linked to

selected CEA sequences result in the desired CEA-specific regulation.

The CEA sequence of Seq IDs 1 and 2 were computer analyzed for characterized consensus sequences which have been associated with gene regulation. Currently not enough is known about transcriptional regulatory sequences to accurately predict by sequence alone whether a sequence will be functional. However, computer searches for characterized consensus sequences can help identify transcriptional regulatory sequences in uncharacterized sequences since many enhancers and promoters consist of unique combinations and spatial alignments of several characterized consensus sequences as well as other sequences. Since not all transcriptional regulatory sequences have been identified and not all sequences that are identical to characterized consensus sequences are functional, such a computer analysis can only suggest possible regions of DNA that may be functionally important for gene regulation.

Some examples of the consensus sequences that are present in the CEA sequence are shown in Figure 3 . Four copies of a lysozymal silencer consensus sequences have been found in the CEA sequence. Inclusion of one or more copies of this consensus sequence in the molecular chimera can help optimize CEA-specific expression. A cluster of topoisomerase II cleavage consensus identified approximately 4-5 kb upstream of the CEA transcriptional start suggest that this region of CEA sequence may contain important transcriptional regulatory signals that may help optimize CEA-specific expression.

The first fragment of CEA genomic sequence analyzed for transcriptional activity extends from -299 to +69, but it is appreciated by those skilled in the art that other fragments are tested in order to isolate a TRS that directs

strong expression in CEA-positive cells but little expression in CEA-negative cells. As diagrammed in Figure 4 the 943 bp SmaI-HindIII fragment of plasmid 39-5-5 was subcloned into the SmaI-HindIII sites of vector pBS+ (Statagene Cloning Systems) creating plasmid 96-11. Single-stranded DNA was rescued from cultures of XL1-blue 96-11 using an M13 helper virus by standard techniques. Oligonucleotide CR70, 5'-CCTGGAACTCAAGCTTGAATTCTCCACAGAGGAGG-3' (SEQ ID NO: 5), was used as a primer for oligonucleotide-directed mutagenesis to introduce HindIII and EcoRI restriction sites at +65. Clone 109-3 was isolated from the mutagenesis reaction and was verified by restriction and DNA sequence analysis to contain the desired changes in the DNA sequence. CEA genomic sequences -299 to +69, original numbering Figure 3, were isolated from 109-3 as a 381 bp EcoRI/HindIII fragment. Plasmid pRc/CMV (Invitrogen Corporation, San Diego, CA, USA) was restricted with AatII and HindIII and the 4.5 kb fragment was isolated from low melting point agarose by standard techniques. The 4.5 kb fragment of pRc/CMV was ligated to the 381 bp fragment of 109-3 using T4 DNA ligase. During this ligation the compatible HindIII ends of the two different restriction fragments were ligated. Subsequently the ligation reaction was supplemented with the four deoxynucleotides, dATP, dCTP, dGTP, and dTTP, and T4 DNA polymerase in order to blunt the non-compatible AatII and EcoRI ends. After incubating, phenol extracting, and ethanol precipitating the reaction, the DNAs were again incubated with T4 DNA ligase. The resulting plasmid, pCR92, allows the insertion of any desired coding sequence into the unique HindIII site downstream of the CEA TRS, upstream from a polyadenylation site and linked to a dominant selectable marker. The coding sequence for CD or other desirable effector or reporter gene, when inserted in the correct orientation into the HindIII site, are transcriptionally regulated by the CEA sequences and are preferably expressed in cells

that express CEA but not in cells that do not express CEA.

In order to determine the optimized CEA TRS other reporter gene constructs containing various fragments of CEA genomic sequences are made by standard techniques from DNA isolated from any of the CEA genomic clones (Figures 1, 2, 4, and 5). DNA fragments extending from the HindIII site introduced at position +65 (original numbering Figure 3A) and numerous different upstream sites are isolated and cloned into the unique HindIII site in plasmid pSVOALdelta5' (De Wet, J.R., <u>et</u> <u>al</u>. Mol. Cell. Biol., <u>7</u>, 725-737 (1987) which is herein incorporated by reference in its entirety) or any similar reporter gene plasmid to construct luciferase reporter gene constructs, Figures 4 and 5. These and similar constructs are used in transient expression assays performed in several CEA-positive and CEA-negative cell lines to determine a strong, CEA-positive cell-type specific TRS. Figures 5B, 5C, and 5D show the results obtained from several CEA/luciferase reporter constructs. The optimized TRS is used to regulate the expression of CD or other desirable gene in a cell-type specific pattern in order to be able to specifically kill cancer cells. The desirable expression cassette is added to a retroviral shuttle vector to aid in delivery of the expression cassette to cancerous tissue.

Strains containing plasmids 39-5-5 and 39-5-2 were deposited at the ATCC under the Budapest Treaty with Accession No. 68904 and 68905, respectively. A strain containing plasmid pCR92 was deposited with the ATCC under the Budapest Treaty with Accession No. 68914. A strain containing plasmid pCR145 was deposited at the ATCC under the Budapest Treaty with Accession No. 69460.

<u>B) Cloning and isolation of the E. coli gene encoding cytosine deaminase (CD)</u>

The cloning, sequencing and expression of *E. coli* CD has already been published (Austin & Huber, Molecular Pharmacology, 43, 380 - 387 (1993) the disclosure of which is incorporated herein by reference). A positive genetic selection was designed for the cloning of the *cod*A gene from *E. coli*. The selection took advantage of the fact that *E. coli* is only able to metabolize cytosine via CD. Based on this, an *E. coli* strain was constructed that could only utilize cytosine as a pyrimidine source when cytosine deaminase was provided *in trans*. This strain, BA101, contains a deletion of the *cod*AB operon and a mutation in the *pyr*F gene. The strain was created by transducing a *pyr*F mutation (obtained from the E. *coli* strain X82 (*E. coli* Genetic Stock Center, New Haven, CT, USA)) into the strain MBM7007 (W. Dallas, Burroughs Wellcome Co., NC, USA) which carried a deletion of the chromosome from *lac* to *arg*F. The *pyr*F mutation confers a pyrimidine requirement on the strain, BA101. In addition, the strain is unable to metabolize cytosine due to the *cod*AB deletion. Thus, BA101 is able to grow on minimal medium supplemented with uracil but is unable to utilize cytosine as the sole pyrimidine source.

The construction of BA101 provided a means for positive selection of DNA fragments encoding. The strain, BA101, was transformed with plasmids carrying inserts from the *E. coli* chromosome and the transformants were selected for growth on minimal medium supplemented with cytosine. Using this approach, the transformants were screened for the ability to metabolize cytosine indicating the presence of a DNA fragment encoding CD. Several sources of DNA could be used for the cloning of the *cod*A gene: 1) a library of the *E. coli* chromosome could be purchased commercially (for example from Clontech, Palo Alto, CA, USA or Stratagene, La Jolla, CA, USA) and screened; 2) chromosomal DNA could be isolated from *E. coli*, digested with various restriction enzymes and ligated and plasmid

DNA with compatible ends before screening; and/or 3) bacteriophage lambda clones containing mapped *E. coli* chromosomal DNA inserts could be screened.

Bacteriophage lambda clones (Y. Kohara, National Institute of Genetics, Japan) containing DNA inserts spanning the 6-8 minute region of the *E. coli* chromosome were screened for the ability to provide transient complementation of the *codA* defect. Two clones, 137 and 138 were identified in this manner. Large-scale preparations of DNA from these clones were isolated from 500 ml cultures. Restriction enzymes were used to generate DNA fragments ranging in size from 10-12 kilobases. The enzymes used were *EcoRI*, *EcoRI* and *BamHI*, and *EcoRI* and *HindIII*. DNA fragments of the desired size were isolated from preparative agarose gels by electroelution. The isolated fragments were ligated to pBR322 (Gibco BRL, Gaithersburg, MD, USA) with compatible ends. The resulting ligation reactions were used to transform the E. *coli* strain, DH5α (Gibco BRL, Gaithersburg, MD, USA). This step was used to amplify the recombinant plasmids resulting from the ligation reactions. The plasmid DNA preparations isolated from the ampicillin-resistant DH5α transformants were digested with the appropriate restriction enzymes to verify the presence of insert DNA. The isolated plasmid DNA was used to transform BA101. The transformed cells were selected for resistance to ampicillin and for the ability to metabolize cytosine. Two clones were isolated pEA001 and pEA002. The plasmid pEA001 contains an approximately 10.8 kb *EcoRI-BamHI* insert while pEA002 contains an approximately 11.5 kb *EcoRI-HindIII* insert. The isolated plasmids were used to transform BA101 to ensure that the ability to metabolize cytosine was the result of the plasmid and not due to a spontaneous chromosomal mutation.

A physical map of the pEA001 DNA insert was generated

using restriction enzymes. Deletion derivatives of pEA001 were constructed based on this restriction map. The resulting plasmids were screened for the ability to allow BA101 to metabolize cytosine. Using this approach, the *cod*A gene was localized to a 4.8 kb *Eco*RI-*Bgl*II fragment. The presence of *cod*A within these inserts was verified by enzymatic assays for CD activity. In addition, cell extracts prepared for enzymatic assay were also examined by polyacrylamide gel electrophoresis. Cell extracts that were positive for enzymatic activity also had a protein band migrating with an apparent molecular weight of 52,000.

The DNA sequence of both strands was determined for a 1634 bp fragment. The sequence determination began at the *Pst*I site and extended to *Pvu*II site thus including the *cod*A coding domain. An open reading frame of 1283 nucleotides was identified. The thirty amino terminal amino acids were confirmed by protein sequencing. Additional internal amino acid sequences were generated from CNBr-digestion of gel-purified CD.

A 200 bp PstI fragment was isolated that spanned the translational start codon of *cod*A. This fragment was cloned into pBS$^+$. Single-stranded DNA was isolated from 30 ml culture and mutanized using a custom oligonuclotide BA22 purchased from Synthecell Inc., Rockville, MD, USA and the oligonucleotide-directed mutagenesis kit (Amersham, Arlington Heights, IL, USA). The base changes result in the introduction of an *Hind*III restriction enzyme site for joining of CD with CEA TRS and in a translational start codon of ATG rather than GTG. The resulting 90 bp *Hind*III-*Pst*I fragment is isolated and ligated with the remainder of the cytosine deaminase gene. The chimeric CEA TRS/cytosine deaminase gene is created by ligating the *Hind*III-*Pvu*II cytosine deaminase-containing DNA fragment with th CEA TRS sequences.

The strain BA101 and the plasmids, pEA001 and pEA003, were deposited with ATCC under the Budapest Treaty with Accession Nos. 55299, 68916, and 68915 respectively.

C) Construction of transcriptional regulatory sequence of carcinoembryonic antigen/cytosine deaminase molecular chimera

A 1508 bp HindIII/PvuII fragment containing the coding sequence for cytosine deaminase is isolated from the plasmid containing the full length CD gene of Example 1B that has been altered to contain a HindIII restriction site just 5′ of the initiation codon. Plasmid pCR92 contains CEA sequences -299 to +69 immediately 5′ to a unique HindIII restriction site and a polyadenylation signal 3′ to a unique ApaI restriction site (Example 1A, Figure 4). pCR92 is linearised with ApaI, the ends are blunted using dNTPs and T4 DNA polymerase, and subsequently digested with HindIII. The pCR92 HindIII/ApaI fragment is ligated to the 1508 bp HindIII/PvuII fragment containing cytosine deaminase. Plasmid pCEA-1/codA, containing CD inserted in the appropriate orientation relative to the CEA TRS and polyadenylation signal is identified by restriction enzyme and DNA sequence analysis.

The optimized CEA-specific TRS, the coding sequence for CD with an ATG translation start, and a suitable polyadenylation signal are joined together using standard molecular biology techniques. The resulting plasmid, containing CD inserted in the appropriate orientation relative to the optimized CEA specific TRS and a polyadenylation signal is identified by restriction enzyme and DNA sequence analysis.

## Example 2

Construction of a retroviral shuttle vector construct

containing the molecular chimera of Example 1

The retroviral shuttle vector pL-CEA-1/codA is constructed by ligating a suitable restriction fragment containing the optimized CEA TRS/codA molecular chimera including the polyadenylation signal into an appropriate retroviral shuttle vector, such as N2(XM5) linearised at the XhoI site, using standard molecular biology techniques. The retroviral shuttle vector pL-CEA-1/codA is characterized by restriction endonuclease mapping and partial DNA sequencing.

Example 3

Virus Production of Retroviral Constructs of Example 3

The retroviral shuttle construct described in Example 2 is placed into an appropriate packaging cell line, such as PA317, by electroporation or infection. Drug resistant colonies, such as those resistant to G418 when using shuttle vectors containing the NEO gene, are single cell cloned by the limiting dilution method, analyzed by Southern blots, and titred in NIH 3T3 cells to identify the highest producer of full-length virus.

Example 4

Further data on the CEA TRS

In addition to the plasmids shown in figure 5B, the following combinations of regions have proved particularly advantageous at high level expression of the reporter gene in the system described in Example 1A:

pCR177:
(-14.5kb to -10.6kb) + (-6.1kb to -3.9kb) + (-299b to +69b)
pCR176:
(-13.6kb to -10.6kb) + (-6.1kb to -3.9kb) + (-299b to +69b)
pCR165:
(-3.9kb to -6.1kb) + (4x -90b to +69b)
pCR168:
(-13.6kb to -10.6kb) + (4x -90b to +69b).

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: The Wellcome Foundation Limited
(B) STREET: Unicorn House, 160 Euston Road
(C) CITY: London
(E) COUNTRY: G.B.
(F) POSTAL CODE (ZIP): NW1 2BP

(ii) TITLE OF INVENTION: Transcriptional Regulatory Sequence

(iii) NUMBER OF SEQUENCES: 5

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11288 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

AAGCTTAAAA CCCAATGGAT TGACAACATC AAGAGTTGGA ACAAGTGGAC ATGGAGATGT 60

TACTTGTGGA AATTTAGATG TGTTCAGCTA TCGGGCAGGA GAATCTGTGT CAAATTCCAG 120

CATGGTTCAG AAGAATCAAA AAGTGTCACA GTCCAAATGT GCAACAGTGC AGGGGATAAA 180

ACTGTGGTGC ATTCAAACTG AGGGATATTT TGGAACATGA GAAAGGAAGG GATTGCTGCT 240

GCACAGAACA TGGATGATCT CACACATAGA GTTGAAAGAA AGGAGTCAAT CGCAGAATAG 300

AAAATGATCA CTAATTCCAC CTCTATAAAG TTTCCAAGAG GAAAACCCAA TTCTGCTGCT 360

AGAGATCAGA ATGGAGGTGA CCTGTGCCTT GCAATGGCTG TGAGGGTCAC GGGAGTGTCA 420

CTTAGTGCAG GCAATGTGCC GTATCTTAAT CTGGGCAGGG CTTTCATGAG CACATAGGAA 480

TGCAGACATT ACTGCTGTGT TCATTTTACT TCACCGGAAA AGAAGAATAA AATCAGCCGG 540

GCGCGGTGGC TCACGCCTGT AATCCCAGCA CTTTAGAAGG CTGAGGTGGG CAGATTACTT 600

GAGGTCAGGA GTTCAAGACC ACCCTGGCCA ATATGGTGAA ACCCCGGCTC TACTAAAAAT 660

ACAAAAATTA GCTGGGCATG GTGGTGCGCG CCTGTAATCC CAGCTACTCG GGAGGCTGAG 720

GCTGGACAAT TGCTTGGACC CAGGAAGCAG AGGTTGCAGT GAGCCAAGAT TGTGCCACTG 780
CACTCCAGCT TGGGCAACAG AGCCAGACTC TGTAAAAAAA AAAAAAAAAA AAAAAAAAAG 840
AAAGAAAGAA AAAGAAAAGA AAGTATAAAA TCTCTTTGGG TTAACAAAAA AAGATCCACA 900
AAACAAACAC CAGCTCTTAT CAAACTTACA CAACTCTGCC AGAGAACAGG AAACACAAAT 960
ACTCATTAAC TCACTTTTGT GGCAATAAAA CCTTCATGTC AAAAGGAGAC CAGGACACAA 1020
TGAGGAAGTA AAACTGCAGG CCCTACTTGG GTGCAGAGAG GGAAAATCCA CAAATAAAAC 1080
ATTACCAGAA GGAGCTAAGA TTTACTGCAT TGAGTTCATT CCCCAGGTAT GCAAGGTGAT 1140
TTTAACACCT GAAAATCAAT CATTGCCTTT ACTACATAGA CAGATTAGCT AGAAAAAAAT 1200
TACAACTAGC AGAACAGAAG CAATTTGGCC TTCCTAAAAT TCCACATCAT ATCATCATGA 1260
TGGAGACAGT GCAGACGCCA ATGACAATAA AAAGAGGGAC CTCCGTCACC CGGTAAACAT 1320
GTCCACACAG CTCCAGCAAG CACCCGTCTT CCCAGTGAAT CACTGTAACC TCCCCTTTAA 1380
TCAGCCCCAG GCAAGGCTGC CTGCGATGGC CACACAGGCT CCAACCCGTG GGCCTCAACC 1440
TCCCGCAGAG GCTCTCCTTT GGCCACCCCA TGGGGAGAGC ATGAGGACAG GGCAGAGCCC 1500
TCTGATGCCC ACACATGGCA GGAGCTGACG CCAGAGCCAT GGGGGCTGGA GAGCAGAGCT 1560
GCTGGGGTCA GAGCTTCCTG AGGACACCCA GGCCTAAGGG AAGGCAGCTC CCTGGATGGG 1620
GGCAACCAGG CTCCGGGCTC CAACCTCAGA GCCCGCATGG GAGGAGCCAG CACTCTAGGC 1680
CTTTCCTAGG GTGACTCTGA GGGGACCCTG ACACGACAGG ATCGCTGAAT GCACCCGAGA 1740
TGAAGGGGCC ACCACGGGAC CCTGCTCTCG TGGCAGATCA GGAGAGAGTG GGACACCATG 1800
CCAGGCCCCC ATGGCATGGC TGCGACTGAC CCAGGCCACT CCCCTGCATG CATCAGCCTC 1860
GGTAAGTCAC ATGACCAAGC CCAGGACCAA TGTGGAAGGA AGGAAACAGC ATCCCCTTTA 1920
GTGATGGAAC CCAAGGTCAG TGCAAAGAGA GGCCATGAGC AGTTAGGAAG GGTGGTCCAA 1980
CCTACAGCAC AAACCATCAT CTATCATAAG TAGAAGCCCT GCTCCATGAC CCCTGCATTT 2040
AAATAAACGT TTGTTAAATG AGTCAAATTC CCTCACCATG AGAGCTCACC TGTGTGTAGG 2100
CCCATCACAC ACACAAACAC ACACACACAC ACACACACAC ACACACACAC ACACAGGGAA 2160
AGTGCAGGAT CCTGGACAGC ACCAGGCAGG CTTCACAGGC AGAGCAAACA GCGTGAATGA 2220
CCCATGCAGT GCCCTGGGCC CCATCAGCTC AGAGACCCTG TGAGGGCTGA GATGGGGCTA 2280
GGCAGGGGAG AGACTTAGAG AGGGTGGGGC CTCCAGGGAG GGGGCTGCAG GGAGCTGGGT 2340
ACTGCCCTCC AGGGAGGGGG CTGCAGGGAG CTGGGTACTG CCCTCCAGGG AGGGGGCTGC 2400
AGGGAGCTGG GTACTGCCCT CCAGGGAGGG GGCTGCAGGG AGCTGGGTAC TGCCCTCCAG 2460
GGAGGGGGCT GCAGGGAGCT GGGTACTGCC CTCCAGGGAG GCAGGAGCAC TGTTCCCAAC 2520
AGAGAGCACA TCTTCCTGCA GCAGCTGCAC AGACACAGGA GCCCCCATGA CTGCCCTGGG 2580
CCAGGGTGTG GATTCCAAAT TTCGTGCCCC ATTGGGTGGG ACGGAGGTTG ACCGTGACAT 2640
CCAAGGGGCA TCTGTGATTC CAAACTTAAA CTACTGTGCC TACAAAATAG GAAATAACCC 2700
TACTTTTTCT ACTATCTCAA ATTCCCTAAG CACAAGCTAG CACCCTTTAA ATCAGGAAGT 2760

TCAGTCACTC CTGGGGTCCT CCCATGCCCC CAGTCTGACT TGCAGGTGCA CAGGGTGGCT 2820
GACATCTGTC CTTGCTCCTC CTCTTGGCTC AACTGCCGCC CCTCCTGGGG GTGACTGATG 2880
GTCAGGACAA GGGATCCTAG AGCTGGCCCC ATGATTGACA GGAAGGCAGG ACTTGGCCTC 2940
CATTCTGAAG ACTAGGGGTG TCAAGAGAGC TGGGCATCCC ACAGAGCTGC ACAAGATGAC 3000
GCGGACAGAG GGTGACACAG GGCTCAGGGC TTCAGACGGG TCGGGAGGCT CAGCTGAGAG 3060
TTCAGGGACA GACCTGAGGA GCCTCAGTGG GAAAAGAAGC ACTGAAGTGG GAAGTTCTGG 3120
AATGTTCTGG ACAAGCCTGA GTGCTCTAAG GAAATGCTCC CACCCCGATG TAGCCTGCAG 3180
CACTGGACGG TCTGTGTACC TCCCCGCTGC CCATCCTCTC ACAGCCCCCG CCTCTAGGGA 3240
CACAACTCCT GCCCTAACAT GCATCTTTCC TGTCTCATTC CACACAAAAG GGCCTCTGGG 3300
GTCCCTGTTC TGCATTGCAA GGAGTGGAGG TCACGTTCCC ACAGACCACC CAGCAACAGG 3360
GTCCTATGGA GGTGCGGTCA GGAGGATCAC ACGTCCCCCC ATGCCCAGGG GACTGACTCT 3420
GGGGGTGATG GATTGGCCTG GAGGCCACTG GTCCCCTCTG TCCCTGAGGG GAATCTGCAC 3480
CCTGGAGGCT GCCACATCCC TCCTGATTCT TTCAGCTGAG GGCCCTTCTT GAAATCCCAG 3540
GGAGGACTCA ACCCCCACTG GGAAAGGCCC AGTGTGGACG GTTCCACAGC AGCCCAGCTA 3600
AGGCCCTTGG ACACAGATCC TGAGTGAGAG AACCTTTAGG GACACAGGTG CACGGCCATG 3660
TCCCCAGTGC CCACACAGAG CAGGGGCATC TGGACCCTGA GTGTGTAGCT CCCGCGACTG 3720
AACCCAGCCC TTCCCCAATG ACGTGACCCC TGGGGTGGCT CCAGGTCTCC AGTCCATGCC 3780
ACCAAAATCT CCAGATTGAG GGTCCTCCCT TGAGTCCCTG ATGCCTGTCC AGGAGCTGCC 3840
CCCTGAGCAA ATCTAGAGTG CAGAGGGCTG GGATTGTGGC AGTAAAAGCA GCCACATTTG 3900
TCTCAGGAAG GAAAGGGAGG ACATGAGCTC CAGGAAGGGC GATGGCGTCC TCTAGTGGGC 3960
GCCTCCTGTT AATGAGCAAA AAGGGGCCAG GAGAGTTGAG AGATCAGGGC TGGCCTTGGA 4020
CTAAGGCTCA GATGGAGAGG ACTGAGGTGC AAAGAGGGGG CTGAAGTAGG GGAGTGGTCG 4080
GGAGAGATGG GAGGAGCAGG TAAGGGGAAG CCCCAGGGAG GCCGGGGGAG GGTACAGCAG 4140
AGCTCTCCAC TCCTCAGCAT TGACATTTGG GGTGGTCGTG CTAGTGGGGT TCTGTAAGTT 4200
GTAGGGTGTT CAGCACCATC TGGGGACTCT ACCCACTAAA TGCCAGCAGG ACTCCCTCCC 4260
CAAGCTCTAA CAACCAACAA TGTCTCCAGA CTTTCCAAAT GTCCCCTGGA GAGCAAAATT 4320
GCTTCTGGCA GAATCACTGA TCTACGTCAG TCTCTAAAAG TGACTCATCA GCGAAATCCT 4380
TCACCTCTTG GGAGAAGAAT CACAAGTGTG AGAGGGGTAG AAACTGCAGA CTTCAAAATC 4440
TTTCCAAAAG AGTTTTACTT AATCAGCAGT TTGATGTCCC AGGAGAAGAT ACATTTAGAG 4500
TGTTTAGAGT TGATGCCACA TGGCTGCCTG TACCTCACAG CAGGAGCAGA GTGGGTTTTC 4560
CAAGGGCCTG TAACCACAAC TGGAATGACA CTCACTGGGT TACATTACAA AGTGGAATGT 4620
GGGGAATTCT GTAGACTTTG GGAAGGGAAA TGTATGACGT GAGCCCACAG CCTAAGGCAG 4680
TGGACAGTCC ACTTTGAGGC TCTCACCATC TAGGAGACAT CTCAGCCATG AACATAGCCA 4740
CATCTGTCAT TAGAAAACAT GTTTTATTAA GAGGAAAAAT CTAGGCTAGA AGTGCTTTAT 4800

GCTCTTTTTT CTCTTTATGT TCAAATTCAT ATACTTTTAG ATCATTCCTT AAAGAAGAAT 4860
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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3774 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CCCTGTGATC TCCAGGACAG CTCAGTCTCC GTCCAATCTC 40

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTTTCCTGAG TGATGTCTGT GTGCAATG 28

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

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CLAIMS:

1. A DNA molecule comprising the carcinoembryonic antigen (CEA) transcriptional regulatory sequence (TRS) but without associated CEA coding sequence.

2. A molecular chimaera comprising a CEA TRS and a DNA sequence operatively linked thereto encoding a heterologous enzyme.

3. A molecular chimaera according to claim 2 wherein the heterologous enzyme is capable of catalysing the production of an agent cytoxic or cytostatic to $CEA^+$ cells.

4. A molecular chimaera according to claim 3 wherein the heterologous enzyme is cytosine deaminase (CD).

5. A molecular chimaera according to any of claims 2 to 4 wherein the CEA TRS and the sequence encoding a heterologous enzyme are in an expression cassette.

6. A molecular chimaera according to claim 5 which comprises DNA sequence of the coding sequence of the gene coding for the heterologous enzyme and additionally includes an appropriate polyadenylation sequence which is linked downstream in a 3' position and in proper orientation to the CEA TRS.

7. A retroviral shuttle vector comprising a molecular chimaera according to any of claims 2 to 6.

8. A retroviral shuttle vector according to claim 7 comprising a DNA sequence comprising a 5' viral LTR sequence, a cis acting psi encapsidation sequence, the molecular chimaera and a 3' viral LTR sequence.

9. A retroviral shuttle vector according to claim 8 based on Moloney murine leukaemia virus.

10. A retroviral shuttle vector according to any of claims 7 to 9 which is a SIN vector.

11. An infective virion comprising a retroviral shuttle vector according to any of claims 7 to 10, the vector being encapsidated within viral proteins to create an artificial, infective, replication defective, retrovirus.

12. A packaging cell line comprising a retroviral shuttle vector according to any of claims 7 to 10.

13. A pharmaceutical composition comprising an infective virion according to claim 11 or packaging cell line according to claim 12 together with a pharmaceutically acceptable carrier.

14. Use of CEA TRS for targeting expression of a heterologous enzyme to $CEA^+$ cells.

15. Use according to claim 14 wherein the heterologous enzyme is capable of catalysing the production of an agent cytotoxic or cytostatic to $CEA^+$ cells.

16. Use according to claim 15 wherein the heterologous enzyme is CD.

17. A DNA milecule according to claim 1 which comprises one or more of the following sequence regions of the CEA gene in either orientation:
about -299b to about +69b, more preferably about -90b to about +69b;
-14.4kb to -10.6kb, preferably -13.6kb to -10.6kb;

-6.1kb to -3.8kb.

18. A molecular chimaera according to any of claims 2 to 6, retroviral shuttle vector according to any claims 7 to 10, packaging cell line according to claim 12 or composition according to claim 13 wherein the CEA TRS comprises one or more of the following sequence regions of the CEA gene in either orientation:

about -299b to about +69b, more preferably about -90b to about +60b;

-14.4kb to -10.6kb, preferably -13.6kb to -10.6kb;

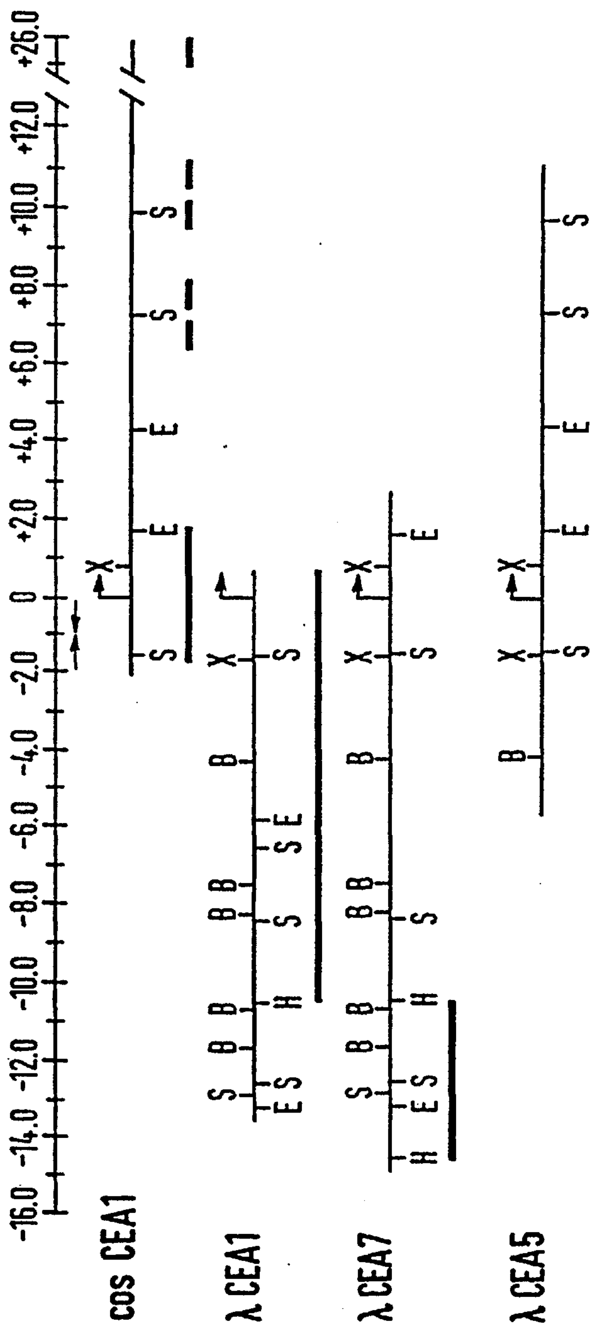
-6.1kb to -3.8kb.

19. Use according to any of claims 14 to 16 wherein the CEA TRS comprises one or more of the following sequence regions of the CEA gene in either orientation:

about -199b to about +69b, more preferably about -90b to about +69b;

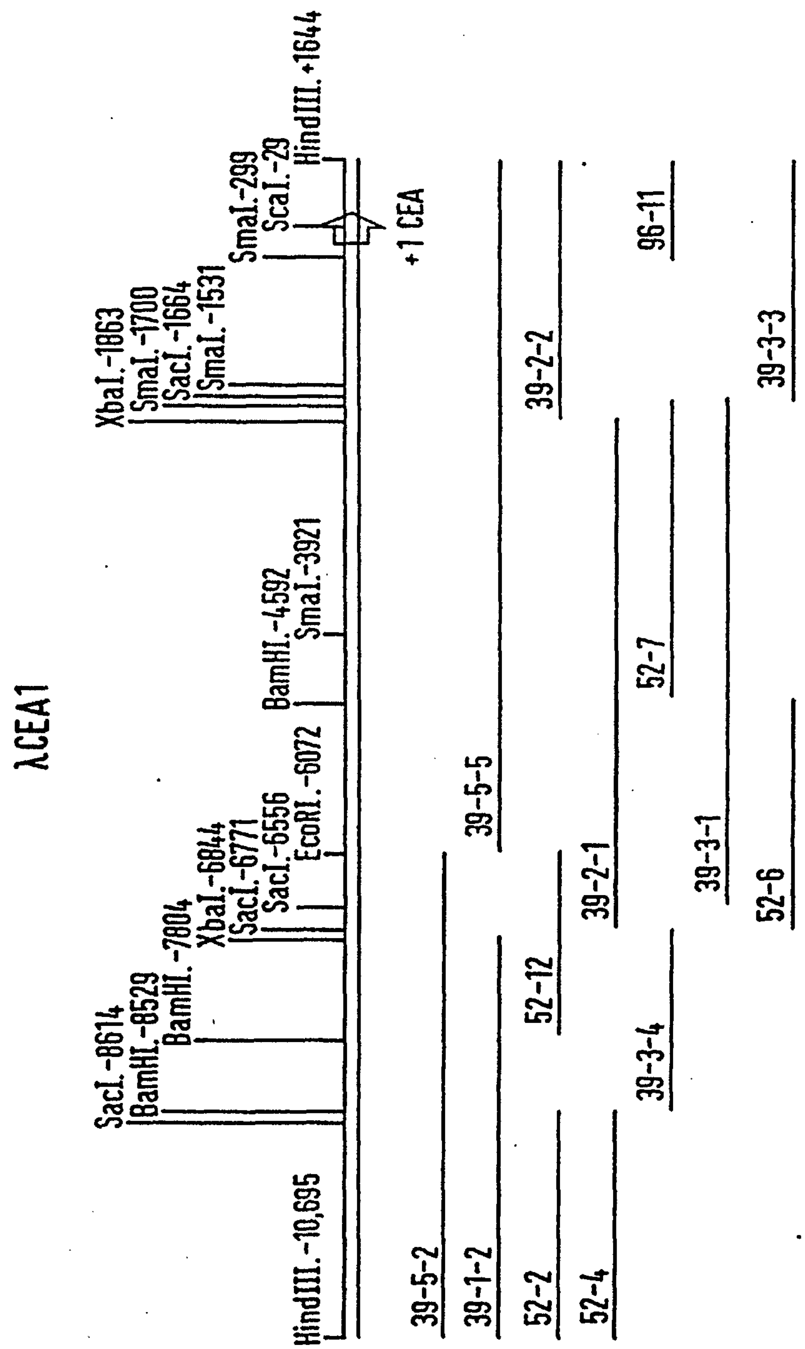
-14.4kb to -10.6kb, preferably -13.6kb to -10.6kb;

-6.1kb to -3.8kb.




cos CEA1 MAP AND SEQUENCE FROM SCHREWE, et.al. Mol Cell Biol 10:2738, 1990

Fig. 1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SacI.-8614
BamHI.-8529
BamHI.-7804
XbaI.-6844
SacI.-6771
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EcoRI.-6072
BamHI.-4592
SmaI.-3921
XbaI.-1863
SmaI.-1700
SacI.-1664
SmaI.-1531
SmaI.-299
ScaI.-29
HindIII.+1644
+1 CEA
39-5-2
39-1-2
52-2
52-4
39-5-5
52-12
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39-2-1
39-3-4
96-11
52-7
39-3-1
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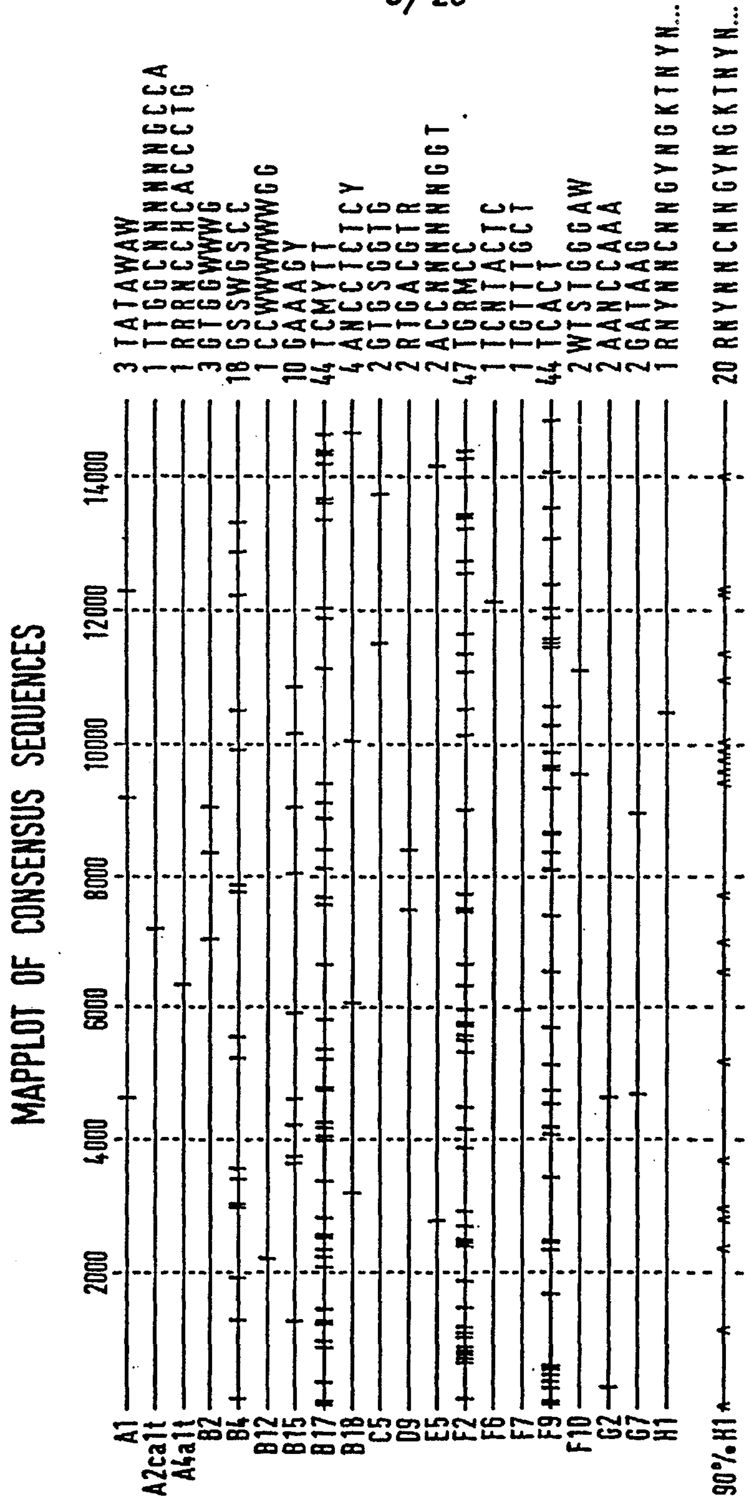
52-6

Fig. 2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14000
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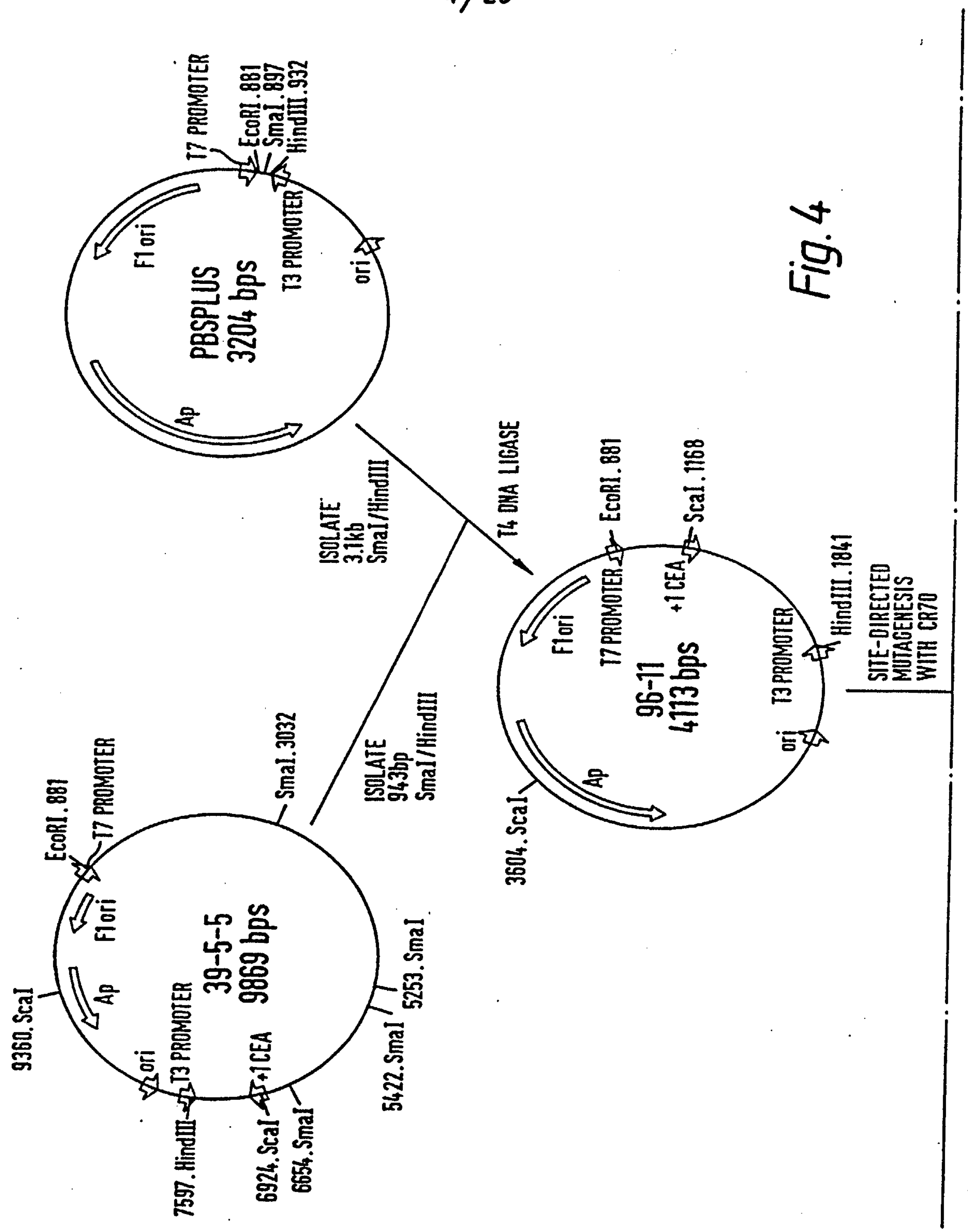
A2calt
A4alt
B2
B4
B12
B15
B17
B18
C5
D9
E5
F2
F6
F7
F9
F10
G2
G7
H1
90% H1

Fig.3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EcoRI.881
SmaI.897
HindIII.932
T3 PROMOTER
F1 ori
PBSPLUS
3204 bps
ori
Ap
T4 DNA LIGASE
ISOLATE
3.1kb
SmaI/HindIII
EcoRI.881
ScaI.1168
T7 PROMOTER
+1 CEA
F1 ori
96-11
4113 bps
HindIII.1841
T3 PROMOTER
SITE-DIRECTED
MUTAGENESIS
WITH CR70
ori
Ap
3604.ScaI
SmaI.3032
ISOLATE
943bp
SmaI/HindIII
EcoRI.881
T7 PROMOTER
F1 ori
39-5-5
9869 bps
Ap
T3 PROMOTER
9360.ScaI
5253.SmaI
ori
+1 CEA
5422.SmaI
7597.HindIII
6924.ScaI
6654.SmaI

Fig. 4

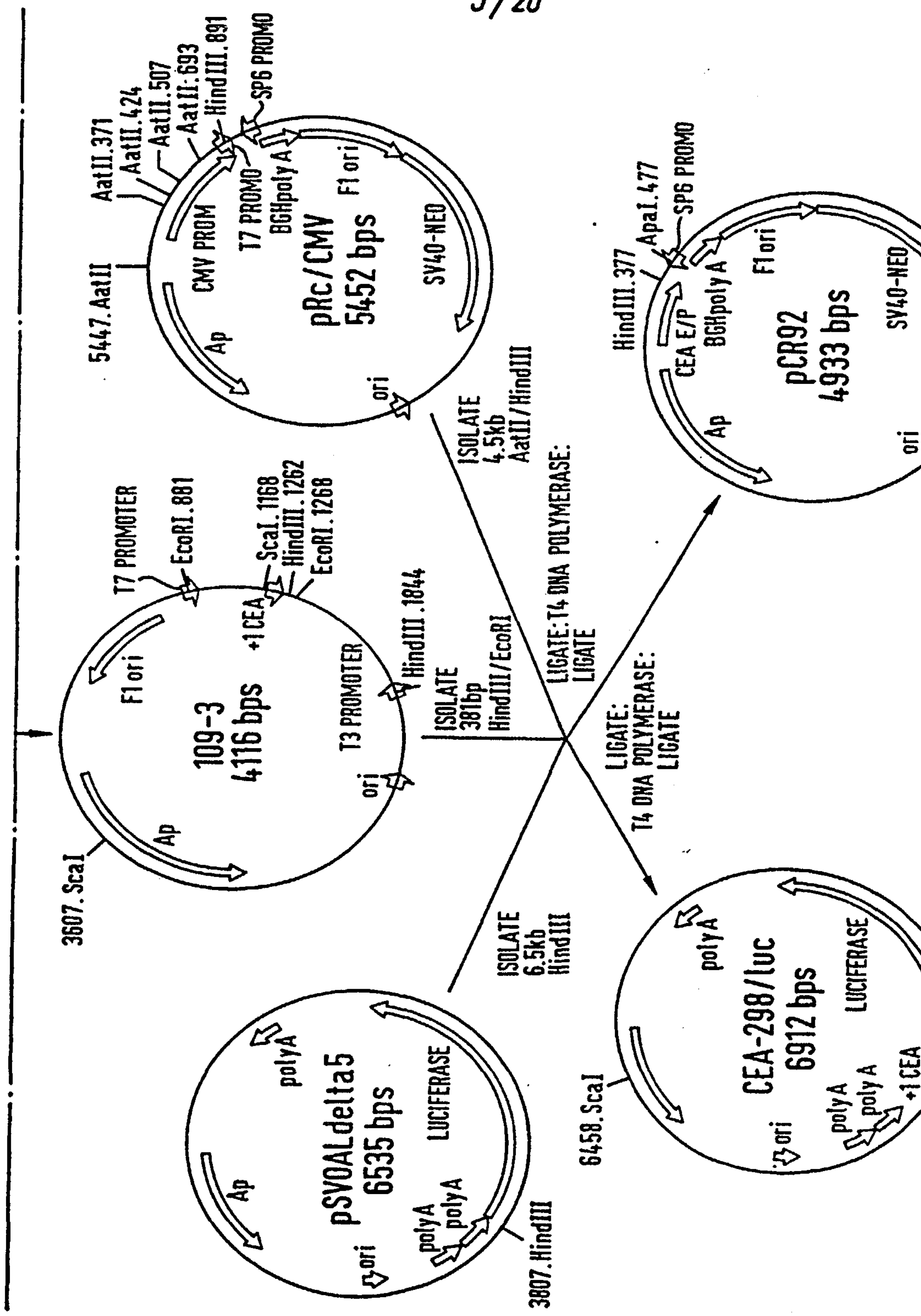
pRc/CMV
5452 bps
5447.AatII
AatII.371
AatII.424
AatII.507
AatII:693
HindIII.891
SP6 PROMO
CMV PROM
T7 PROMO
BGHpoly A
F1 ori
SV40-NEO
Ap
ori
109-3
4116 bps
T7 PROMOTER
EcoRI.881
ScaI.1168
HindIII.1262
EcoRI.1268
+1 CEA
HindIII.1844
F1 ori
T3 PROMOTER
ori
Ap
3607.ScaI
ISOLATE
4.5kb
AatII/HindIII
LIGATE: T4 DNA POLYMERASE:
LIGATE
ISOLATE
381bp
HindIII/EcoRI
LIGATE:
T4 DNA POLYMERASE:
LIGATE
pCR92
4933 bps
HindIII.377
ApaI.477
SP6 PROMO
CEA E/P
BGHpoly A
F1 ori
SV40-NEO
Ap
ori
ISOLATE
6.5kb
HindIII
pSVOALdelta5
6535 bps
polyA
LUCIFERASE
Ap
ori
polyA
polyA
3807.HindIII
CEA-298/luc
6912 bps
polyA
LUCIFERASE
6458.ScaI
ori
polyA
poly A
+1 CEA
3897.ScaI
3803.HindIII

Fig.4(cont.)

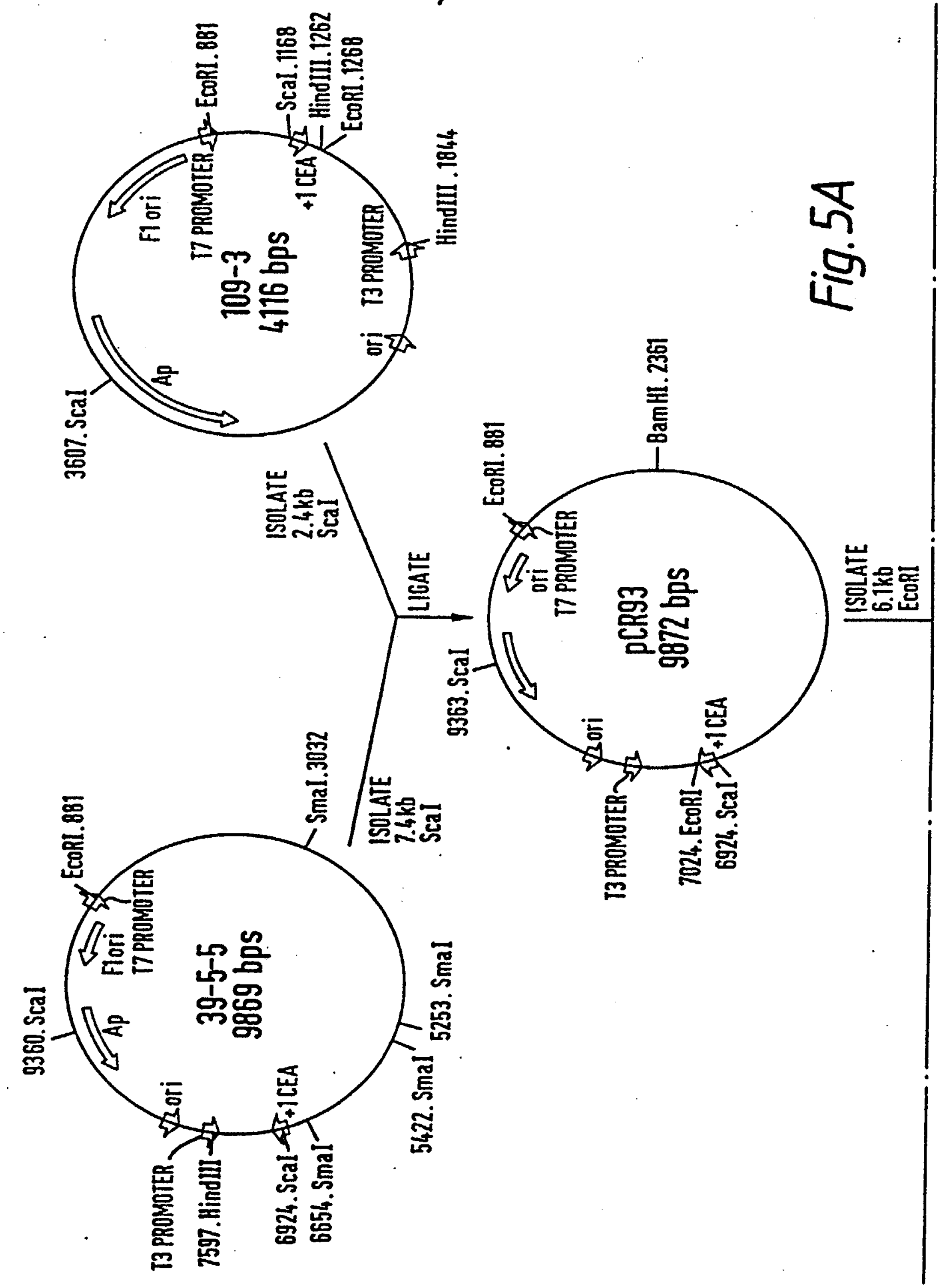
109-3
4116 bps
EcoRI.881
ScaI.1168
HindIII.1262
EcoRI.1268
HindIII.1844
T7 PROMOTER
F1 ori
+1 CEA
T3 PROMOTER
ori
Ap
3607.ScaI
ISOLATE
2.4kb
ScaI
LIGATE
pCR93
9872 bps
BamHI.2361
EcoRI.881
T7 PROMOTER
ori
9363.ScaI
ori
+1 CEA
T3 PROMOTER
7024.EcoRI
6924.ScaI
ISOLATE
6.1kb
EcoRI
39-5-5
9869 bps
SmaI.3032
ISOLATE
7.4kb
ScaI
EcoRI.881
T7 PROMOTER
F1ori
Ap
9360.ScaI
ori
+1 CEA
T3 PROMOTER
7597.HindIII
6924.ScaI
6654.SmaI
5422.SmaI
5253.SmaI

Fig.5A

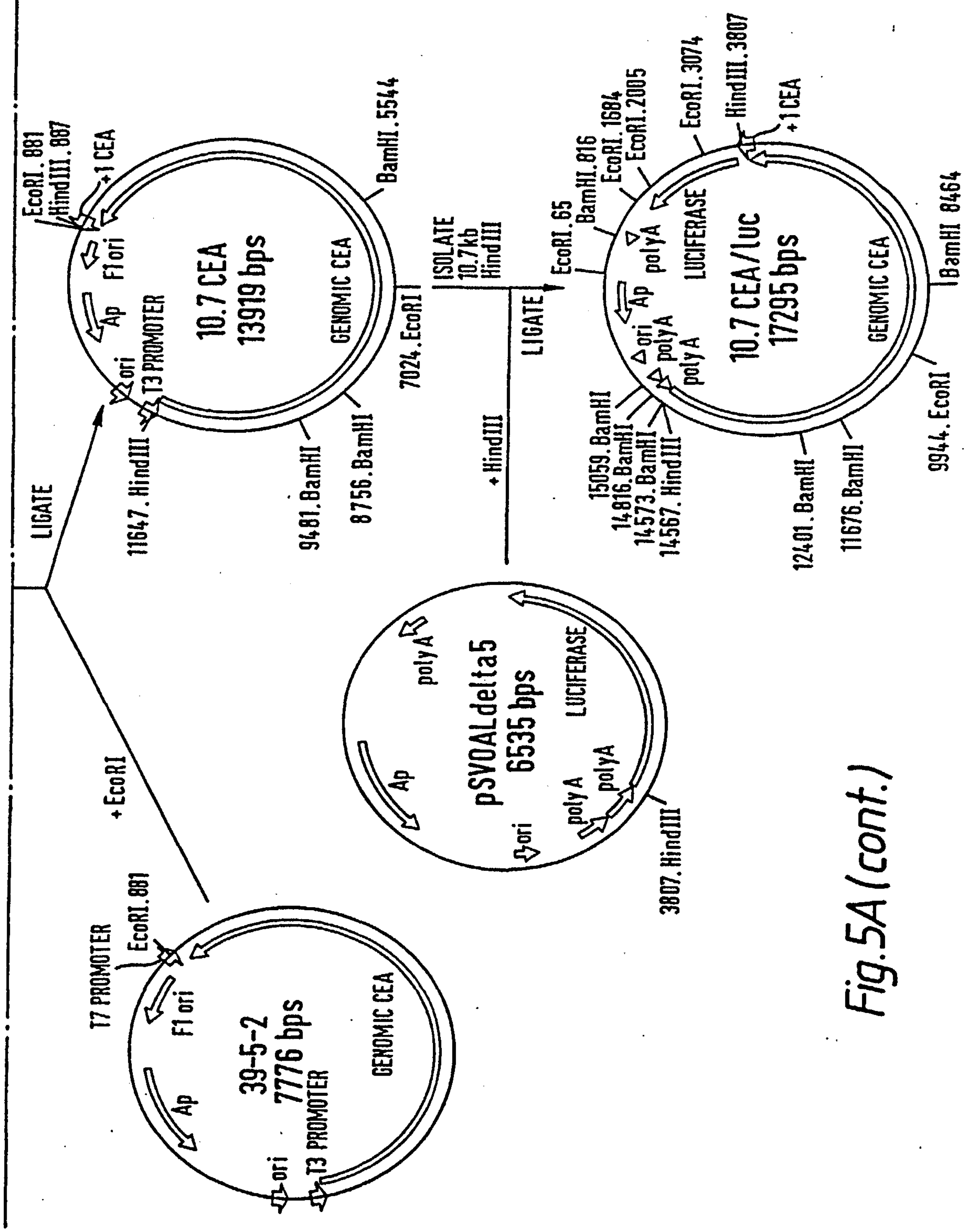
39-5-2
7776 bps
T7 PROMOTER
EcoRI.881
F1 ori
Ap
ori
T3 PROMOTER
GENOMIC CEA
+EcoRI
LIGATE
10.7 CEA
13919 bps
EcoRI.881
HindIII.887
+1 CEA
BamHI.5544
7024.EcoRI
8756.BamHI
9481.BamHI
11647.HindIII
GENOMIC CEA
pSVOALdelta5
6535 bps
LUCIFERASE
polyA
3807.HindIII
+HindIII
ISOLATE
10.7kb
HindIII
LIGATE
10.7 CEA/luc
17295 bps
EcoRI.65
BamHI.816
EcoRI.1684
EcoRI.2005
EcoRI.3074
HindIII.3807
BamHI 8464
9944.EcoRI
11676.BamHI
12401.BamHI
14567.HindIII
14573.BamHI
14816.BamHI
15059.BamHI

Fig.5A (cont.)

| Plasmid | CEA Coordinates |
|---|---|
| pCR113 | (-299 to +69) |
| pCR105 | (-1664 to +69) |
| pCR145 | (-14462 to -10691)+(-299 to +69) |
| pCR148 | (-89 to -40)+(-90 to +69) |
| pCR158 | [3 X (-89 to -40)]+(-90 to +69) |
| pCR136 | (-3919 to -6071) + (-299 to +69) |
| pCR137 | (-6071 to -3919) + (-299 to +69) |
| pCR162 | (-13579 to -10691)+(-89 to -40)+(-90 to +69) |
| pCR163 | (-10691 to -13579)+(-89 to -40)+(-90 to +69) |

Fig.5B

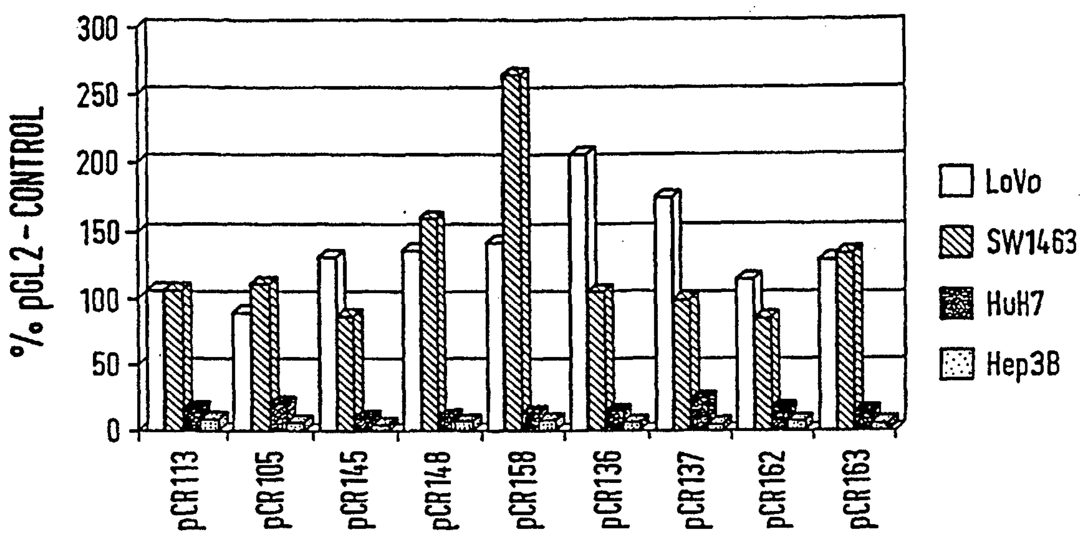
300
250
200
150
100
50
0
% pGL2-CONTROL
LoVo
SW1463
HuH7
Hep3B
pCR113
pCR105
pCR145
pCR148
pCR158
pCR136
pCR137
pCR162
pCR163

Fig.5C

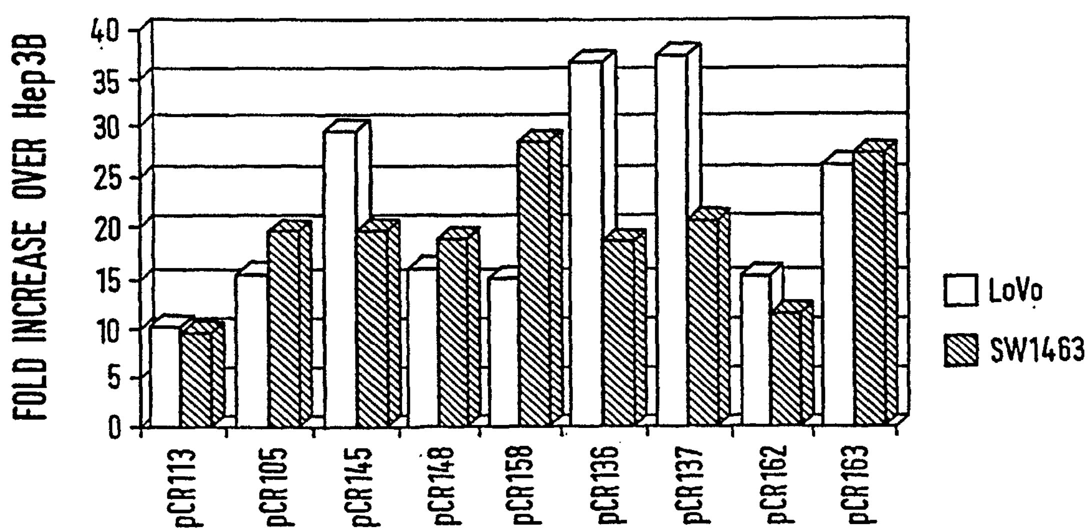
40
35
30
25
20
15
10
5
0
FOLD INCREASE OVER Hep3B
LoVo
SW1463
pCR113
pCR105
pCR145
pCR148
pCR158
pCR136
pCR137
pCR162
pCR163

Fig.5D

-14463 AAGCTTTTTA GTGCTTTAGA CAGTGAGCTG GTCTGTCTAA CCCAAGTGAC CTGGGCTC
-14403 TACTCAGCCC CAGAAGTGAA GGGTGAAGCT GGGTGGAGCC AAACCAGGCA AGCCTACC
-14343 CAGGGCTCCC AGTGGCCTGA GAACCATTGG ACCCAGGACC CATTACTTCT AGGGTAAG
-14283 AGGTACAAAC ACCAGATCCA ACCATGGTCT GGGGGGACAG CTGTCAAATG CCTAAAAA
-14223 TACCTGGGAG AGGAGCAGGC AAACTATCAC TGCCCCAGGT TCTCTGAACA GAAACAGA
-14163 GGCAACCCAA AGTCCAAATC CAGGTGAGCA GGTGCACCAA ATGCCCAGAG ATATGACG
-14103 GCAAGAAGTG AAGGAACCAC CCCTGCATCA AATGTTTTGC ATGGGAAGGA GAAGGGGG
-14043 GCTCATGTTC CCAATCCAGG AGAATGCATT TGGGATCTGC CTTCTTCTCA CTCCTTGG
-13983 AGCAAGACTA AGCAACCAGG ACTCTGGATT TGGGGAAAGA CGTTTATTTG TGGAGGCC
-13923 TGATGACAAT CCCACGAGGG CCTAGGTGAA GAGGGCAGGA AGGCTCGAGA CACTGGGG
-13863 TGAGTGAAAA CCACACCCAT GATCTGCACC ACCCATGGAT GCTCCTTCAT TGCTCACC
-13803 TCTGTTGATA TCAGATGGCC CCATTTTCTG TACCTTCACA GAAGGACACA GGCTAGGG
-13743 TGTGCATGGC CTTCATCCCC GGGGCCATGT GAGGACAGCA GGTGGGAAAG ATCATGGG
-13683 CTCCTGGGTC CTGCAGGGCC AGAACATTCA TCACCCATAC TGACCTCCTA GATGGGAA
-13623 GCTTCCCTGG GGCTGGGCCA ACGGGGCCTG GGCAGGGGAG AAAGGACGTC AGGGGACA
-13563 GAGGAAGGGT CATCGAGACC CAGCCTGGAA GGTTCTTGTC TCTGACCATC CAGGATTT
-13503 TTCCCTGCAT CTACCTTTGG TCATTTTCCC TCAGCAATGA CCAGCTCTGC TTCCTGAT
-13443 CAGCCTCCCA CCCTGGACAC AGCACCCCAG TCCCTGGCCC GGCTGCATCC ACCCAATA
-13383 CTGATAACCC AGGACCCATT ACTTCTAGGG TAAGGAGGGT CCAGGAGACA GAAGCTGA
-13323 AAAGGTCTGA AGAAGTCACA TCTGTCCTGG CCAGAGGGGA AAAACCATCA GATGCTGA
-13263 CAGGAGAATG TTGACCCAGG AAAGGGACCG AGGACCCAAG AAAGGAGTCA GACCACCA
-13203 GTTTGCCTGA GAGGAAGGAT CAAGGCCCCG AGGGAAAGCA GGGCTGGCTG CATGTGCA

Fig. 6 (1/11)

-13143 ACACTGGTGG GGCATATGTG TCTTAGATTC TCCCTGAATT CAGTGTCCCT GCCATGGC
-13083 GACTCTCTAC TCAGGCCTGG ACATGCTGAA ATAGGACAAT GGCCTTGTCC TCTCTCCC
-13023 CCATTTGGCA AGAGACATAA AGGACATTCC AGGACATGCC TTCCTGGGAG GTCCAGGT
-12963 TCTGTCTCAC ACCTCAGGGA CTGTAGTTAC TGCATCAGCC ATGGTAGGTG CTGATCTC
-12903 CCAGCCTGTC CAGGCCCTTC CACTCTCCAC TTTGTGACCA TGTCCAGGAC CACCCCTC
-12843 ATCCTGAGCC TGCAAATACC CCCTTGCTGG GTGGGTGGAT TCAGTAAACA GTGAGCTC
-12783 ATCCAGCCCC CAGAGCCACC TCTGTCACCT TCCTGCTGGG CATCATCCCA CCTTCACA
-12723 CACTAAAGAG CATGGGGAGA CCTGGCTAGC TGGGTTTCTG CATCACAAAG AAAATAAT
-12663 CCCAGGTTCG GATTCCCAGG GCTCTGTATG TGGAGCTGAC AGACCTGAGG CCAGGAGA
-12603 GCAGAGGTCA GCCCTAGGGA GGGTGGGTCA TCCACCCAGG GGACAGGGGT GCACCAGC
-12543 TGCTACTGAA AGGGCCTCCC CAGGACAGCG CCATCAGCCC TGCCTGAGAG CTTTGCTA
-12483 CAGCAGTCAG AGGAGGCCAT GGCAGTGGCT GAGCTCCTGC TCCAGGCCCC AACAGACC
-12423 ACCAACAGCA CAATGCAGTC CTTCCCCAAC GTCACAGGTC ACCAAAGGGA AACTGAGG
-12363 CTACCTAACC TTAGAGCCAT CAGGGGAGAT AACAGCCCAA TTTCCCAAAC AGGCCAGT
-12303 CAATCCCATG ACAATGACCT CTCTGCTCTC ATTCTTCCCA AAATAGGACG CTGATTCT
-12243 CCCACCATGG ATTTCTCCCT TGTCCCGGGA GCCTTTTCTG CCCCCTATGA TCTGGGCA
-12183 CCTGACACAC ACCTCCTCTC TGGTGACATA TCAGGGTCCC TCACTGTCAA GCAGTCCA
-12123 AAGGACAGAA CCTTGGACAG CGCCCATCTC AGCTTCACCC TTCCTCCTTC ACAGGGTT
-12063 GGGCAAAGAA TAAATGGCAG AGGCCAGTGA GCCCAGAGAT GGTGACAGGC AGTGACCC
-12003 GGGCAGATGC CTGGAGCAGG AGCTGGCGGG GCCACAGGGA GAAGGTGATG CAGGAAGG
-11943 AACCCAGAAA TGGGCAGGAA AGGAGGACAC AGGCTCTGTG GGGCTGCAGC CCAGGGTT
-11883 ACTATGAGTG TGAAGCCATC TCAGCAAGTA AGGCCAGGTC CCATGAACAA GAGTGGGA
-11823 ACGTGGCTTC CTGCTCTGTA TATGGGGTGG GGGATTCCAT GCCCCATAGA ACCAGATG

Fig. 6 (2/11)

| | | | | | | |
|---|---|---|---|---|---|---|
| -11763 | CGGGGTTCAG | ATGGAGAAGG | AGCAGGACAG | GGGATCCCCA | GGATAGGAGG | ACCCCAGT |
| -11703 | CCCCACCCAG | GCAGGTGACT | GATGAATGGG | CATGCAGGGT | CCTCCTGGGC | TGGGCTCT |
| -11643 | CTTTGTCCCT | CAGGATTCCT | TGAAGGAACA | TCCGGAAGCC | GACCACATCT | ACCTGGTG |
| -11583 | TTCTGGGGAG | TCCATGTAAA | GCCAGGAGCT | TGTGTTGCTA | GGAGGGGTCA | TGGCATGT |
| -11523 | TGGGGGCACC | AAAGAGAGAA | ACCTGAGGGC | AGGCAGGACC | TGGTCTGAGG | AGGCATGG |
| -11463 | GCCCAGATGG | GGAGATGGAT | GTCAGGAAAG | GCTGCCCCAT | CAGGGAGGGT | GATAGCAA |
| -11403 | GGGGGTCTGT | GGGAGTGGGC | ACGTGGGATT | CCCTGGGCTC | TGCCAAGTTC | CCTCCCAT |
| -11343 | TCACAACCTG | GGGACACTGC | CCATGAAGGG | GCGCCTTTGC | CCAGCCAGAT | GCTGCTGG |
| -11283 | CTGCCCATCC | ACTACCCTCT | CTGCTCCAGC | CACTCTGGGT | CTTTCTCCAG | ATGCCCTG |
| -11223 | CAGCCCTGGC | CTGGGCCTGT | CCCCTGAGAG | GTGTTGGGAG | AAGCTGAGTC | TCTGGGGA |
| -11163 | CTCTCATCAG | AGTCTGAAAG | GCACATCAGG | AAACATCCCT | GGTCTCCAGG | ACTAGGCA |
| -11103 | GAGGAAAGGG | CCCCAGCTCC | TCCCTTTGCC | ACTGAGAGGG | TCGACCCTGG | GTGGCCAC |
| -11043 | TGACTTCTGC | GTCTGTCCCA | GTCACCCTGA | AACCACAACA | AAACCCCAGC | CCCAGACC |
| -10983 | GCAGGTACAA | TACATGTGGG | GACAGTCTGT | ACCCAGGGGA | AGCCAGTTCT | CTCTTCCT |
| -10923 | GAGACCGGGC | CTCAGGGCTG | TGCCCGGGGC | AGGCGGGGGC | AGCACGTGCC | TGTCCTTG |
| -10863 | AACTCGGGAC | CTTAAGGGTC | TCTGCTCTGT | GAGGCACAGC | AAGGATCCTT | CTGTCCAG |
| -10803 | ATGAAAGCAG | CTCCTGCCCC | TCCTCTGACC | TCTTCCTCCT | TCCCAAATCT | CAACCAAC |
| -10743 | ATAGGTGTTT | CAAATCTCAT | CATCAAATCT | TCATCCATCC | ACATGAGAAA | GCTTAAAA |
| -10683 | CAATGGATTG | ACAACATCAA | GAGTTGGAAC | AAGTGGACAT | GGAGATGTTA | CTTGTGGA |
| -10623 | TTTAGATGTG | TTCAGCTATC | GGGCAGGAGA | ATCTGTGTCA | AATTCCAGCA | TGGTTCAG |
| -10563 | GAATCAAAAA | GTGTCACAGT | CCAAATGTGC | AACAGTGCAG | GGGATAAAAC | TGTGGTGC |
| -10503 | TCAAACTGAG | GGATATTTTG | GAACATGAGA | AAGGAAGGGA | TTGCTGCTGC | ACAGAACA |
| -10443 | GATGATCTCA | CACATAGAGT | TGAAAGAAAG | GAGTCAATCG | CAGAATAGAA | AATGATCA |

Fig. 6 (3/11)

| | | | | | | |
|---|---|---|---|---|---|---|
| -10383 | AATTCCACCT | CTATAAAGTT | TCCAAGAGGA | AAACCCAATT | CTGCTGCTAG | AGATCAGA |
| -10323 | GGAGGTGACC | TGTGCCTTGC | AATGGCTGTG | AGGGTCACGG | GAGTGTCACT | TAGTGCAG |
| -10263 | AATGTGCCGT | ATCTTAATCT | GGGCAGGGCT | TTCATGAGCA | CATAGGAATG | CAGACATT |
| -10203 | TGCTGTGTTC | ATTTTACTTC | ACCGGAAAAG | AAGAATAAAA | TCAGCCGGGC | GCGGTGGC |
| -10143 | ACGCCTGTAA | TCCCAGCACT | TTAGAAGGCT | GAGGTGGGCA | GATTACTTGA | GGTCAGGA |
| -10083 | TCAAGACCAC | CCTGGCCAAT | ATGGTGAAAC | CCCGGCTCTA | CTAAAAATAC | AAAAATTA |
| -10023 | TGGGCATGGT | GGTGCGCGCC | TGTAATCCCA | GCTACTCGGG | AGGCTGAGGC | TGGACAAT |
| -9963 | CTTGGACCCA | GGAAGCAGAG | GTTGCAGTGA | GCCAAGATTG | TGCCACTGCA | CTCCAGCT |
| -9903 | GGCAACAGAG | CCAGACTCTG | TAAAAAAAAA | AAAAAAAAAA | AAAAAAAGAA | AGAAAGAA |
| -9843 | AGAAAAGAAA | GTATAAAATC | TCTTTGGGTT | AACAAAAAAA | GATCCACAAA | ACAAACAC |
| -9783 | GCTCTTATCA | AACTTACACA | ACTCTGCCAG | AGAACAGGAA | ACACAAATAC | TCATTAAC |
| -9723 | ACTTTTGTGG | CAATAAAACC | TTCATGTCAA | AAGGAGACCA | GGACACAATG | AGGAAGTA |
| -9663 | ACTGCAGGCC | CTACTTGGGT | GCAGAGAGGG | AAAATCCACA | AATAAAACAT | TACCAGAA |
| -9603 | AGCTAAGATT | TACTGCATTG | AGTTCATTCC | CCAGGTATGC | AAGGTGATTT | TAACACCT |
| -9543 | AAATCAATCA | TTGCCTTTAC | TACATAGACA | GATTAGCTAG | AAAAAAATTA | CAACTAGC |
| -9483 | AACAGAAGCA | ATTTGGCCTT | CCTAAAATTC | CACATCATAT | CATCATGATG | GAGACAGT |
| -9423 | AGACGCCAAT | GACAATAAAA | AGAGGGACCT | CCGTCACCCG | GTAAACATGT | CCACACAG |
| -9363 | CCAGCAAGCA | CCCGTCTTCC | CAGTGAATCA | CTGTAACCTC | CCCTTTAATC | AGCCCCAG |
| -9303 | AAGGCTGCCT | GCGATGGCCA | CACAGGCTCC | AACCCGTGGG | CCTCAACCTC | CCGCAGAG |
| -9243 | TCTCCTTTGG | CCACCCCATG | GGGAGAGCAT | GAGGACAGGG | CAGAGCCCTC | TGATGCCC |
| -9183 | ACATGGCAGG | AGCTGACGCC | AGAGCCATGG | GGGCTGGAGA | GCAGAGCTGC | TGGGGTCA |
| -9123 | GCTTCCTGAG | GACACCCAGG | CCTAAGGGAA | GGCAGCTCCC | TGGATGGGGG | CAACCAGG |
| -9063 | CCGGGCTCCA | ACCTCAGAGC | CCGCATGGGA | GGAGCCAGCA | CTCTAGGCCT | TTCCTAGG |

Fig.6 (4/11)

-9003 GACTCTGAGG GGACCCTGAC ACGACAGGAT CGCTGAATGC ACCCGAGATG AAGGGGCC
-8943 CACGGGACCC TGCTCTCGTG GCAGATCAGG AGAGAGTGGG ACACCATGCC AGGCCCCC
-8883 GGCATGGCTG CGACTGACCC AGGCCACTCC CCTGCATGCA TCAGCCTCGG TAAGTCAC
-8823 GACCAAGCCC AGGACCAATG TGGAAGGAAG GAAACAGCAT CCCCTTTAGT GATGGAAC
-8763 AAGGTCAGTG CAAAGAGAGG CCATGAGCAG TTAGGAAGGG TGGTCCAACC TACAGCAC
-8703 ACCATCATCT ATCATAAGTA GAAGCCCTGC TCCATGACCC CTGCATTTAA ATAAACGT
-8643 GTTAAATGAG TCAAATTCCC TCACCATGAG AGCTCACCTG TGTGTAGGCC CATCACAC
-8583 ACAAACACAC ACACACACAC ACACACACAC ACACACACAC ACAGGGAAAG TGCAGGAT
-8523 TGGACAGCAC CAGGCAGGCT TCACAGGCAG AGCAAACAGC GTGAATGACC CATGCAGT
-8463 CCTGGGCCCC ATCAGCTCAG AGACCCTGTG AGGGCTGAGA TGGGGCTAGG CAGGGGAG
-8403 ACTTAGAGAG GGTGGGGCCT CCAGGGAGGG GGCTGCAGGG AGCTGGGTAC TGCCCTCC
-8343 GGAGGGGGCT GCAGGGAGCT GGGTACTGCC CTCCAGGGAG GGGGCTGCAG GGAGCTGG
-8283 ACTGCCCTCC AGGGAGGGGG CTGCAGGGAG CTGGGTACTG CCCTCCAGGG AGGGGGCT
-8223 AGGGAGCTGG GTACTGCCCT CCAGGGAGGC AGGAGCACTG TTCCCAACAG AGAGCACA
-8163 TTCCTGCAGC AGCTGCACAG ACACAGGAGC CCCCATGACT GCCCTGGGCC AGGGTGTG
-8103 TTCCAAATTT CGTGCCCCAT TGGGTGGGAC GGAGGTTGAC CGTGACATCC AAGGGGCA
-8043 TGTGATTCCA AACTTAAACT ACTGTGCCTA CAAAATAGGA AATAACCCTA CTTTTTCT
-7983 TATCTCAAAT TCCCTAAGCA CAAGCTAGCA CCCTTTAAAT CAGGAAGTTC AGTCACTC
-7923 GGGGTCCTCC CATGCCCCCA GTCTGACTTG CAGGTGCACA GGGTGGCTGA CATCTGTC
-7863 TGCTCCTCCT CTTGGCTCAA CTGCCGCCCC TCCTGGGGGT GACTGATGGT CAGGACAA
-7803 GATCCTAGAG CTGGCCCCAT GATTGACAGG AAGGCAGGAC TTGGCCTCCA TTCTGAAG
-7743 TAGGGGTGTC AAGAGAGCTG GGCATCCCAC AGAGCTGCAC AAGATGACGC GGACAGAG
-7683 TGACACAGGG CTCAGGGCTT CAGACGGGTC GGGAGGCTCA GCTGAGAGTT CAGGGACA

Fig. 6 (5/11)

-7623 CCTGAGGAGC CTCAGTGGGA AAAGAAGCAC TGAAGTGGGA AGTTCTGGAA TGTTCTGG
-7563 AAGCCTGAGT GCTCTAAGGA AATGCTCCCA CCCCGATGTA GCCTGCAGCA CTGGACGG
-7503 TGTGTACCTC CCCGCTGCCC ATCCTCTCAC AGCCCCCGCC TCTAGGGACA CAACTCCT
-7443 CCTAACATGC ATCTTTCCTG TCTCATTCCA CACAAAAGGG CCTCTGGGGT CCCTGTTC
-7383 CATTGCAAGG AGTGGAGGTC ACGTTCCCAC AGACCACCCA GCAACAGGGT CCTATGGA
-7323 TGCGGTCAGG AGGATCACAC GTCCCCCCAT GCCCAGGGGA CTGACTCTGG GGGTGATG
-7263 TTGGCCTGGA GGCCACTGGT CCCCTCTGTC CCTGAGGGGA ATCTGCACCC TGGAGGCT
-7203 CACATCCCTC CTGATTCTTT CAGCTGAGGG CCCTTCTTGA AATCCCAGGG AGGACTCA
-7143 CCCCACTGGG AAAGGCCCAG TGTGGACGGT TCCACAGCAG CCCAGCTAAG GCCCTTGG
-7083 ACAGATCCTG AGTGAGAGAA CCTTTAGGGA CACAGGTGCA CGGCCATGTC CCCAGTGC
-7023 ACACAGAGCA GGGGCATCTG GACCCTGAGT GTGTAGCTCC CGCGACTGAA CCCAGCCC
-6963 CCCCAATGAC GTGACCCCTG GGGTGGCTCC AGGTCTCCAG TCCATGCCAC CAAAATCT
-6903 AGATTGAGGG TCCTCCCTTG AGTCCCTGAT GCCTGTCCAG GAGCTGCCCC CTGAGCAA
-6843 CTAGAGTGCA GAGGGCTGGG ATTGTGGCAG TAAAAGCAGC CACATTTGTC TCAGGAAG
-6783 AAGGGAGGAC ATGAGCTCCA GGAAGGGCGA TGGCGTCCTC TAGTGGGCGC CTCCTGTT
-6723 TGAGCAAAAA GGGGCCAGGA GAGTTGAGAG ATCAGGGCTG GCCTTGGACT AAGGCTCA
-6663 TGGAGAGGAC TGAGGTGCAA AGAGGGGGCT GAAGTAGGGG AGTGGTCGGG AGAGATGG
-6603 GGAGCAGGTA AGGGGAAGCC CCAGGGAGGC CGGGGGAGGG TACAGCAGAG CTCTCCAC
-6543 CTCAGCATTG ACATTTGGGG TGGTCGTGCT AGTGGGGTTC TGTAAGTTGT AGGGTGTT
-6483 GCACCATCTG GGGACTCTAC CCACTAAATG CCAGCAGGAC TCCCTCCCCA AGCTCTAA
-6423 ACCAACAATG TCTCCAGACT TTCCAAATGT CCCCTGGAGA GCAAAATTGC TTCTGGCA
-6363 ATCACTGATC TACGTCAGTC TCTAAAAGTG ACTCATCAGC GAAATCCTTC ACCTCTTG
-6303 AGAAGAATCA CAAGTGTGAG AGGGGTAGAA ACTGCAGACT TCAAAATCTT TCCAAAAG

Fig.6 (6/11)

-6243 TTTTACTTAA TCAGCAGTTT GATGTCCCAG GAGAAGATAC ATTTAGAGTG TTTAGAGT

-6183 ATGCCACATG GCTGCCTGTA CCTCACAGCA GGAGCAGAGT GGGTTTTCCA AGGGCCTG

-6123 ACCACAACTG GAATGACACT CACTGGGTTA CATTACAAAG TGGAATGTGG GGAATTCT

-6063 AGACTTTGGG AAGGGAAATG TATGACGTGA GCCCACAGCC TAAGGCAGTG GACAGTCC

-6003 TTTGAGGCTC TCACCATCTA GGAGACATCT CAGCCATGAA CATAGCCACA TCTGTCAT

-5943 GAAAACATGT TTTATTAAGA GGAAAAATCT AGGCTAGAAG TGCTTTATGC TCTTTTTT

-5883 CTTTATGTTC AAATTCATAT ACTTTTAGAT CATTCCTTAA AGAAGAATCT ATCCCCCT

-5823 GTAAATGTTA TCACTGACTG GATAGTGTTG GTGTCTCACT CCCAACCCCT GTGTGGTG

-5763 AGTGCCCTGC TTCCCCAGCC CTGGGCCCTC TCTGATTCCT GAGAGCTTTG GGTGCTCC

-5703 CATTAGGAGG AAGAGAGGAA GGGTGTTTTT AATATTCTCA CCATTCACCC ATCCACCT

-5643 TAGACACTGG GAAGAATCAG TTGCCCACTC TTGGATTTGA TCCTCGAATT AATGACCT

-5583 ATTTCTGTCC CTTGTCCATT TCAACAATGT GACAGGCCTA AGAGGTGCCT TCTCCATG

-5523 ATTTTTGAGG AGAAGGTTCT CAAGATAAGT TTTCTCACAC CTCTTTGAAT TACCTCCA

-5463 TGTGTCCCCA TCACCATTAC CAGCAGCATT TGGACCCTTT TTCTGTTAGT CAGATGCT

-5403 CCACCTCTTG AGGGTGTATA CTGTATGCTC TCTACACAGG AATATGCAGA GGAAATAG

-5343 AAAGGGAAAT CGCATTACTA TTCAGAGAGA AGAAGACCTT TATGTGAATG AATGAGAG

-5283 TAAAATCCTA AGAGAGCCCA TATAAAATTA TTACCAGTGC TAAAACTACA AAAGTTAC

-5223 TAACAGTAAA CTAGAATAAT AAAACATGCA TCACAGTTGC TGGTAAAGCT AAATCAGA

-5163 TTTTTTTCTT AGAAAAAGCA TTCCATGTGT GTTGCAGTGA TGACAGGAGT GCCCTTCA

-5103 CAATATGCTG CCTGTAATTT TTGTTCCCTG GCAGAATGTA TTGTCTTTTC TCCCTTTA

-5043 TCTTAAATGC AAAACTAAAG GCAGCTCCTG GGCCCCCTCC CCAAAGTCAG CTGCCTGC

-4983 CCAGCCCCAC GAAGAGCAGA GGCCTGAGCT TCCCTGGTCA AAATAGGGGG CTAGGGAG

-4923 TAACCTTGCT CGATAAAGCT GTGTTCCCAG AATGTCGCTC CTGTTCCCAG GGGCACCA

Fig. 6 (7/11)

-4863 CTGGAGGGTG GTGAGCCTCA CTGGTGGCCT GATGCTTACC TTGTGCCCTC ACACCAGT
-4803 TCACTGGAAC CTTGAACACT TGGCTGTCGC CCGGATCTGC AGATGTCAAG AACTTCTG
-4743 AGTCAAATTA CTGCCCACTT CTCCAGGGCA GATACCTGTG AACATCCAAA ACCATGCC
-4683 AGAACCCTGC CTGGGGTCTA CAACACATAT GGACTGTGAG CACCAAGTCC AGCCCTGA
-4623 CTGTGACCAC CTGCCAAGAT GCCCCTAACT GGGATCCACC AATCACTGCA CATGGCAG
-4563 AGCGAGGCTT GGAGGTGCTT CGCCACAAGG CAGCCCCAAT TTGCTGGGAG TTTCTTGG
-4503 CCTGGTAGTG GTGAGGAGCC TTGGGACCCT CAGGATTACT CCCCTTAAGC ATAGTGGG
-4443 CCCTTCTGCA TCCCCAGCAG GTGCCCCGCT CTTCAGAGCC TCTCTCTCTG AGGTTTAC
-4383 AGACCCCTGC ACCAATGAGA CCATGCTGAA GCCTCAGAGA GAGAGATGGA GCTTTGAC
-4323 GGAGCCGCTC TTCCTTGAGG GCCAGGGCAG GGAAAGCAGG AGGCAGCACC AGGAGTGG
-4263 ACACCAGTGT CTAAGCCCCT GATGAGAACA GGGTGGTCTC TCCCATATGC CCATACCA
-4203 CCTGTGAACA GAATCCTCCT TCTGCAGTGA CAATGTCTGA GAGGACGACA TGTTTCCC
-4143 CCTAACGTGC AGCCATGCCC ATCTACCCAC TGCCTACTGC AGGACAGCAC CAACCCAG
-4083 GCTGGGAAGC TGGGAGAAGA CATGGAATAC CCATGGCTTC TCACCTTCCT CCAGTCCA
-4023 GGGCACCATT TATGCCTAGG ACACCCACCT GCCGGCCCCA GGCTCTTAAG AGTTAGGT
-3963 CCTAGGTGCC TCTGGGAGGC CGAGGCAGGA GAATTGCTTG AACCCGGGAG GCAGAGGT
-3903 CAGTGAGCCG AGATCACACC ACTGCACTCC AGCCTGGGTG ACAGAATGAG ACTCTGTC
-3843 AAAAAAAAAG AGAAAGATAG CATCAGTGGC TACCAAGGGC TAGGGGCAGG GGAAGGTG
-3783 GAGTTAATGA TTAATAGTAT GAAGTTTCTA TGTGAGATGA TGAAAATGTT CTGGAAAA
-3723 AAATATAGTG GTGAGGATGT AGAATATTGT GAATATAATT AACGGCATTT AATTGTAC
-3663 TTAACATGAT TAATGTGGCA TATTTTATCT TATGTATTTG ACTACATCCA AGAAACAC
-3603 GGAGAGGGAA AGCCCACCAT GTAAAATACA CCCACCCTAA TCAGATAGTC CTCATTGT
-3543 CCAGGTACAG GCCCCTCATG ACCTGCACAG GAATAACTAA GGATTTAAGG ACATGAGG

Fig. 6 (8/11)

-3483 TCCCAGCCAA CTGCAGGTGC ACAACATAAA TGTATCTGCA AACAGACTGA GAGTAAAG
-3423 GGGGGCACAA ACCTCAGCAC TGCCAGGACA CACACCCTTC TCGTGGATTC TGACTTTA
-3363 TGACCCGGCC CACTGTCCAG ATCTTGTTGT GGGATTGGGA CAAGGGAGGT CATAAAGC
-3303 GTCCCCAGGG CACTCTGTGT GAGCACACGA GACCTCCCCA CCCCCCCACC GTTAGGTC
-3243 CACACATAGA TCTGACCATT AGGCATTGTG AGGAGGACTC TAGCGCGGGC TCAGGGAT
-3183 CACCAGAGAA TCAGGTACAG AGAGGAAGAC GGGGCTCGAG GAGCTGATGG ATGACACA
-3123 GCAGGGTTCC TGCAGTCCAC AGGTCCAGCT CACCCTGGTG TAGGTGCCCC ATCCCCCT
-3063 TCCAGGCATC CCTGACACAG CTCCCTCCCG GAGCCTCCTC CCAGGTGACA CATCAGGG
-3003 CCTCACTCAA GCTGTCCAGA GAGGGCAGCA CCTTGGACAG CGCCCACCCC ACTTCACT
-2943 TCCTCCCTCA CAGGGCTCAG GGCTCAGGGC TCAAGTCTCA GAACAAATGG CAGAGGCC
-2883 TGAGCCCAGA GATGGTGACA GGGCAATGAT CCAGGGGCAG CTGCCTGAAA CGGGAGCA
-2823 TGAAGCCACA GATGGGAGAA GATGGTTCAG GAAGAAAAAT CCAGGAATGG GCAGGAGA
-2763 AGAGGAGGAC ACAGGCTCTG TGGGGCTGCA GCCCAGGATG GGACTAAGTG TGAAGACA
-2703 TCAGCAGGTG AGGCCAGGTC CCATGAACAG AGAAGCAGCT CCCACCTCCC CTGATGCA
-2643 GACACACAGA GTGTGTGGTG CTGTGCCCCC AGAGTCGGGC TCTCCTGTTC TGGTCCCC
-2583 GGAGTGAGAA GTGAGGTTGA CTTGTCCCTG CTCCTCTCTG CTACCCCAAC ATTCACCT
-2523 TCCTCATGCC CCTCTCTCTC AAATATGATT TGGATCTATG TCCCCGCCCA AATCTCAT
-2463 CAAATTGTAA ACCCCAATGT TGGAGGTGGG GCCTTGTGAG AAGTGATTGG ATAATGCG
-2403 TGGATTTTCT GCTTTGATGC TGTTTCTGTG ATAGAGATCT CACATGATCT GGTTGTTT
-2343 AAGTGTGTAG CACCTCTCCC CTCTCTCTCT CTCTCTCTTA CTCATGCTCT GCCATGTA
-2283 ACGTTCCTGT TTCCCCTTCA CCGTCCAGAA TGATTGTAAG TTTTCTGAGG CCTCCCCA
-2223 AGCAGAAGCC ACTATGCTTC CTGTACAACT GCAGAATGAT GAGCGAATTA AACCTCTT
-2163 CTTTATAAAT TACCCAGTCT CAGGTATTTC TTTATAGCAA TGCGAGGACA GACTAATA

Fig. 6 (9/11)

| | | | | | | |
|---|---|---|---|---|---|---|
| -2103 | ATCTTCTACT | CCCAGATCCC | CGCACACGCT | TAGCCCCAGA | CATCACTGCC | CCTGGGAG |
| -2043 | TGCACAGCGC | AGCCTCCTGC | CGACAAAAGC | AAAGTCACAA | AAGGTGACAA | AAATCTGC |
| -1983 | TTGGGGACAT | CTGATTGTGA | AAGAGGGAGG | ACAGTACACT | TGTAGCCACA | GAGACTGG |
| -1923 | CTCACCGAGC | TGAAACCTGG | TAGCACTTTG | GCATAACATG | TGCATGACCC | GTGTTCAA |
| -1863 | TCTAGAGATC | AGTGTTGAGT | AAAACAGCCT | GGTCTGGGGC | CGCTGCTGTC | CCCACTTC |
| -1803 | TCCTGTCCAC | CAGAGGGCGG | CAGAGTTCCT | CCCACCCTGG | AGCCTCCCCA | GGGGCTGC |
| -1743 | ACCTCCCTCA | GCCGGGCCCA | CAGCCCAGCA | GGGTCCACCC | TCACCCGGGT | CACCTCGG |
| -1683 | CACGTCCTCC | TCGCCCTCCG | AGCTCCTCAC | ACGGACTCTG | TCAGCTCCTC | CCTGCAGC |
| -1623 | ATCGGCCGCC | CACCTGAGGC | TTGTCGGCCG | CCCACTTGAG | GCCTGTCGGC | TGCCCTCT |
| -1563 | AGGCAGCTCC | TGTCCCCTAC | ACCCCCTCCT | TCCCCGGGCT | CAGCTGAAAG | GGCGTCTC |
| -1503 | AGGGCAGCTC | CCTGTGATCT | CCAGGACAGC | TCAGTCTCTC | ACAGGCTCCG | ACGCCCCC |
| -1443 | TGCTGTCACC | TCACAGCCCT | GTCATTACCA | TTAACTCCTC | AGTCCCATGA | AGTTCACT |
| -1383 | GCGCCTGTCT | CCCGGTTACA | GGAAAACTCT | GTGACAGGGA | CCACGTCTGT | CCTGCTCT |
| -1323 | GTGGAATCCC | AGGGCCCAGC | CCAGTGCCTG | ACACGGAACA | GATGCTCCAT | AAATACTG |
| -1263 | TAAATGTGTG | GGAGATCTCT | AAAAAGAAGC | ATATCACCTC | CGTGTGGCCC | CCAGCAGT |
| -1203 | GAGTCTGTTC | CATGTGGACA | CAGGGGCACT | GGCACCAGCA | TGGGAGGAGG | CCAGCAAG |
| -1143 | CCCGCGGCTG | CCCCAGGAAT | GAGGCCTCAA | CCCCCAGAGC | TTCAGAAGGG | AGGACAGA |
| -1083 | CCTGCAGGGA | ATAGATCCTC | CGGCCTGACC | CTGCAGCCTA | ATCCAGAGTT | CAGGGTCA |
| -1023 | TCACACCACG | TCGACCCTGG | TCAGCATCCC | TAGGGCAGTT | CCAGACAAGG | CCGGAGGT |
| -963 | CCTCTTGCCC | TCCAGGGGGT | GACATTGCAC | ACAGACATCA | CTCAGGAAAC | GGATTCCC |
| -903 | GGACAGGAAC | CTGGCTTTGC | TAAGGAAGTG | GAGGTGGAGC | CTGGTTTCCA | TCCCTTGC |
| -843 | CAACAGACCC | TTCTGATCTC | TCCCACATAC | CTGCTCTGTT | CCTTTCTGGG | TCCTATGA |
| -783 | ACCCTGTTCT | GCCAGGGGTC | CCTGTGCAAC | TCCAGACTCC | CTCCTGGTAC | CACCATGG |

Fig. 6 (10/11)

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-723  AAGGTGGGGT GATCACAGGA CAGTCAGCCT CGCAGAGACA GAGACCACCC AGGACTGT
-663  GGGAGAACAT GGACAGGCCC TGAGCCGCAG CTCAGCCAAC AGACACGGAG AGGGAGGG
-603  CCCCTGGAGC CTTCCCCAAG GACAGCAGAG CCCAGAGTCA CCCACCTCCC TCCACCAC
-543  TCCTCTCTTT CCAGGACACA CAAGACACCT CCCCCTCCAC ATGCAGGATC TGGGGACT
-483  TGAGACCTCT GGGCCTGGGT CTCCATCCCT GGGTCAGTGG CGGGGTTGGT GGTACTGG
-423  ACAGAGGGCT GGTCCCTCCC CAGCCACCAC CCAGTGAGCC TTTTTCTAGC CCCCAGAG
-363  ACCTCTGTCA CCTTCCTGTT GGGCATCATC CCACCTTCCC AGAGCCCTGG AGAGCATG
-303  GAGACCCGGG ACCCTGCTGG GTTTCTCTGT CACAAAGGAA AATAATCCCC CTGGTGTG
-243  AGACCCAAGG ACAGAACACA GCAGAGGTCA GCACTGGGGA AGACAGGTTG TCCTCCCA
-183  GGATGGGGGT CCATCCACCT TGCCGAAAAG ATTTGTCTGA GGAACTGAAA ATAGAAGG
-123  AAAAAGAGGA GGGACAAAAG AGGCAGAAAT GAGAGGGGAG GGGACAGAGG ACACCTGA
 -63  AAAGACCACA CCCATGACCC ACGTGATGCT GAGAAGTACT CCTGCCCTAG GAAGAGAC
  -3  AGGGCAGAGG GAGGAAGGAC AGCAGACCAG ACAGTCACAG CAGCCTTGAC AAAACGTT
  57  TGGAACTCAA GCTCTTCTCC ACAGAGGAGG ACAGAGCAGA CAGCAGAGAC CATGGAGT
 117  CCCTCGGCCC CTCCCCACAG ATGGTGCATC CCCTGGCAGA GGCTCCTGCT CACAGGTG
 177  GGGAGGACAA CCTGGGAGAG GGTGGGAGGA GGGAGCTGGG GTCTCCTGGG TAGGACAG
 237  CTGTGAGACG GACAGAGGGC TCCTGTTGGA GCCTGAATAG GGAAGAGGAC ATCAGAGA
 297  GACAGGAGTC ACACCAGAAA AATCAAATTG AACTGGAATT GGAAAGGGGC AGGAAAAC
 357  CAAGAGTTCT ATTTTCCTAG TTAATTGTCA CTGGCCACTA CGTTTTTAAA AATCATAA
 417  ACTGCATCAG ATGACACTTT AAATAAAAAC ATAACCAGGG CATGAAACAC TGTCCTCA
 477  CGCCTACCGC GGACATTGGA AAATAAGCCC CAGGCTGTGG AGGGCCCTGG GAACCCTC
 537  GAACTCATCC ACAGGAATCT GCAGCCTGTC CCAGGCACTG GGGTGCAACC AAGATC
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*Fig.6 (11/11)*

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(74) Agent: STOTT, Michael, John; The Wellcome Foundation Limited, Langley Court, Beckenham, Kent BR3 3BS (GB).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ).

**Published**

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*Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

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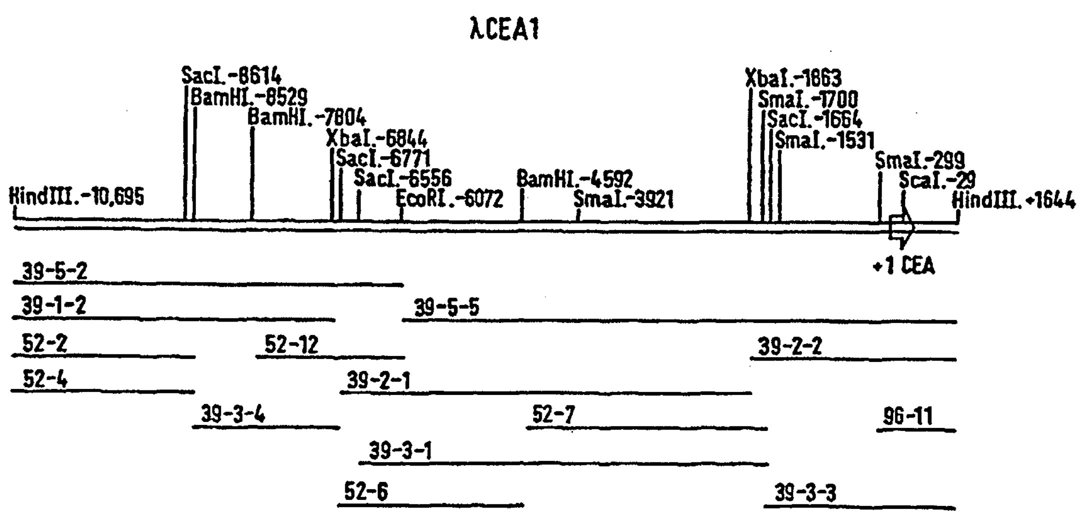


(57) Abstract

The invention relates to the transcriptional regulatory sequence (TRS) of carcinoembryonic antigen (CEA) and molecular chimaera comprising the CEA TRS and DNA encoding a heterologous enzyme. CEA TRS is capable of targeting expression of the heterologous enzyme to CEA+ cells and the heterologous enzyme is preferably an enzyme capable of catalysing the production of an agent cytotoxic or cytostatic to CEA+ cells. For example the enzyme may be cytosine deaminase which is capable of catalysing formation of the cytotoxic compound 5-fluorouracil from the non toxic compound 5-fluorocytosine.

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# INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 94/02546

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/85 A61K48/00 C12N15/86 C12N5/10 C12N7/01

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | DNA SEQUENCE, vol.4, no.3, July 1993, SWITZERLAND pages 185 - 196 RICHARDS, C. A. ET AL. 'The transcriptional control region of the human carcinoembryonic antigen gene: DNA sequence and homology studies' | 1 |
| Y | see the whole document | 2-19 |
| | --- | |
| | -/-- | |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 12 April 1995 | 11-05-1995 |
| Name and mailing address of the ISA<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Authorized officer<br>Chambonnet, F |

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | MOLECULAR AND CELLULAR BIOLOGY, vol.10, no.6, June 1990 pages 2738 - 2748 SCHREWE, H. ET AL. 'Cloning of the complete gene for carcinoembryonic antigen : analysis of its promoter indicates a region conveying cell-type specific expression' cited in the application | 1 |
| Y | see the whole document see page 2739, column 1, line 1 - line 6; figure 4 | 1-19 |
| | --- | |
| P,X | ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, vol.716, 1994 pages 104 - 114 HUBER, B.E. ET AL. 'Virus-Directed Enzyme/Prodrug Therapy (VDEPT) : Selectively engineering drug sensitivity into tumors' | 2-16 |
| Y,P | see the whole document | 2-19 |
| O,X | & Gene therapy for neoplastic diseases Washington, USA june 26-29th 1993 | 3 |
| | --- | |
| X | TRENDS IN BIOTECHNOLOGY., vol.11, no.5, May 1993, CAMBRIDGE GB pages 197 - 201 SIKORA, K. 'Gene therapy for cancer' | 2-5, 14-16 |
| Y | see page 198, column 2, line 6 - page 199, column 1, line 34; figure 2; table 1 | 2-19 |
| | --- | |
| P,Y | GENE THERAPY, vol.1, no.3, May 1994 pages 170 - 175 HARRIS, J. D. ET AL. 'Gene therapy for cancer using tumour-specific prodrug activation' see the whole document | 2-19 |
| | --- | |
| Y | CANCER RESEARCH, vol.53, no.19, 1 October 1993 pages 4619 - 4626 HUBER, B.E. ET AL. 'In vivo antitumor activity of 5-fluorocytosine on human colorectal carcinoma cells genetically modifies to express cytosine deaminase' see the whole document | 2-19 |
| | --- | |
| | -/-- | |

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | CANCER RESEARCH, vol.54, no.20, 15 October 1994 pages 5258 - 5261 TADASHI OSAKI ET AL. 'Gene therapy for carcinoembryonic antigen-producing human lung cancer cells by cell type-specific expression of Herpes Simplex Virus Thymidine Kinase gene' | 2,3,5 |
| Y | see page 5258, column 2, line 16 - line 34 see page 5261, column 1, line 6 - column 2, line 8 | 1-19 |
| A | EP,A,0 415 731 (WELLCOME FOUNDATION LTD.) 6 March 1991 cited in the application see the whole document | 1 |
| A | WO,A,92 15693 (THE WELLCOME FOUNDATION) 17 September 1992 see claims 1-3 | 1 |

1

## INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB94/02546

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 14-16,19, as far as they concern an "in vivo" method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 94/02546

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| EP-A-0415731 | 06-03-91 | AU-B- 647747 | 31-03-94 |
| | | AU-A- 6199190 | 07-03-91 |
| | | AU-B- 6458794 | 08-09-94 |
| | | CN-A- 1050899 | 24-04-91 |
| | | JP-A- 3172189 | 25-07-91 |
| WO-A-9215693 | 17-09-92 | EP-A- 0573479 | 15-12-93 |
| | | JP-T- 6505156 | 16-06-94 |

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